2014

Soil aggregate distribution and turnover affects soil microbial ecology and ecosystem processes in three bioenergy systems

Elizabeth Marie Bach
Iowa State University

Soil aggregate distribution and turnover affects soil microbial ecology and ecosystem processes in three bioenergy systems

by

Elizabeth Marie Bach

A dissertation submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Ecology and Evolutionary Biology

Program of Study Committee:
Kirsten S. Hofmockel, Major Professor
Cynthia A. Cambardella
Richard M. Cruse
Thomas M. Isenhart
Michael L. Thompson
Brian J. Wilsey

Iowa State University

Ames, Iowa

2014

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

This dissertation is the product of many hours work under the guidance, patience, and support of my adviser, Dr. Kirsten Hofmockel. She has allowed me the freedom to explore questions that pushed both of us into new realms of knowledge. In addition, the expertise and support of my committee members Dr. Cynthia Cambardella, Dr. Richard Cruse, Dr. Thomas Isenhart, and Dr. Brian Wilsey has expanded my research questions and my approach to answering them. They have also made the process a lot more fun.

My colleagues in the Hofmockel Lab deserve much credit for teaching and assisting me throughout the years, most especially Sarah Hargreaves and Ryan Williams. The numerous and enthusiastic undergraduate assistants have provide invaluable assistance and kept me excited about coming back to the lab every day. Thank you Eric Asbe, Meghan Bartholomew, Austin Putz, Rebecca Luzbetak, Giselle Narvaez, Elyssa McFarland, Kira Murray, and Erin Frankson! In addition, my fellow EEB and EEOB colleagues have been reliable support in and outside the lab. Thank you Lauren Sullivan, Erik Mudrak, Ali Berens, Michelle Balmer, Mindy Morales, and Clay Williams. Dr. Sara Baer deserves much gratitude for her contributions to the formation of my love for soil ecology and the skills needed to pursue a research career.

My academic achievements in the past four years would not have been possible without the unflagging support of my family, most especially my parents Jeff and Ann Bach. They have instilled curiosity, hard-work, acceptance, and fun into my approach to research and life in general.

ABSTRACT

Soil microorganisms are the drivers of ecosystem biogeochemistry, but the field of soil ecology lags behind other biological disciplines in understanding habitat constraints driving biodiversity and ecosystem functioning of these microorganisms. One approach to characterizing habitat at the micro-scale is to subset soil into naturally occurring physical associations of particles, organic matter, and microbes called aggregates. However, classic methods of separating soil aggregates can drastically change microbial communities and activities. In this dissertation, a methodological approach to isolating soil aggregates for biological analysis is refined and then applied to evaluating microbial activity and fungal community structure in three ecosystems managed for bioenergy production. Subsequently, aggregate-scale processes are scaled out to contrast ecosystem carbon (C) and nitrogen (N) cycling in the three ecosystems.

Direct contrast of soil extracellular enzyme activities within soil aggregates isolated by traditional slaking means, dry sieving, and optimal moisture found wet sieved large macroaggregates (>2000 μm) had four times greater activity than macroaggregates isolated by the other methods. Very high activity in wet-sieved macroaggregates led to mass-proportional sums of enzyme activities to exceed 100% of whole soil measurements. This indicates the wet-sieving procedure induces enzyme activity not observed in whole soil or in dry and optimal moisture aggregates. Although there were few differences in enzyme activity between dry-sieved and optimal moisture aggregates, the additional care taken in optimal moisture sieving, including rapid partial-drying under sterile conditions at 4°C, may be more important in other metrics of soil biological analysis including DNA-based assays.

Using the optimal moisture approach, I contrasted extracellular enzyme activity and C and N resources within aggregates isolated across two growing seasons from three ecosystems managed for bioenergy feedstock production: row-crop continuous corn agroecosystems and reconstructed tallgrass prairie with and without annual inorganic N fertilizer application. Across aggregate fractions, N-acetyl-glucosaminidase (NAG) activity was greatest in large macroaggregates and cellobiohydrolase activity was greatest in microaggregates (<250 μm). Increased NAG activity in large macroaggregates is likely driven by greater total C and N within that fraction. Increased cellobiohydrolase activity in microaggregates may indicate enzymes are largely stabilized on the outside of microaggregates, interacting with substrates in the surrounding pore networks rather than intra-aggregate organic matter. Aggregate turn-over was detected across the two growing seasons and disintegration of large macroaggregates corresponded with peaks in enzyme activity. Release of organic matter with aggregate turn-over may be a driver of these spikes in enzyme activity.

Fungi are major components of soil microbial communities and perform several important decomposition functions. However, there has been less research dedicated to fungal communities and the forces structuring them compared with soil bacteria. I utilized the optimal moisture aggregate isolation approach to evaluate soil fungal communities within soil aggregates from the aforementioned bioenergy cropping systems. Fungal richness was much greater in microaggregates compared with large macroaggregates and proportional sums of aggregate-level richness indicate whole soil sampling approaches are underestimating fungal richness two-fold. However, fungal community structure was affected by ecosystem to greater extent than aggregation. Unfertilized prairies supported

greater abundance of members of the Basidiomycota family Strophariaceae, and genus *Limonomyces*, which were not present in corn or fertilized prairie systems, but were highly abundant in prairie systems. Fertilized prairies contained greater abundance of fungi from the Basidiomycota family Psathyrellaceae and genus Thanatephours as well as Ascomycota family Orbiliaceae and *Trichoderma citrinoviride*. Corn communities were distinguished with greater abundance of unknown Basidiomycota and arbuscular mycorrhizal fungi from the order Glomerales.

Aggregate-scale differences in extracellular enzyme activity and fungal communities contributed to ecosystem-scale differences in C and N cycling between the unfertilized prairie, fertilized prairie, and corn systems. Whole soil analysis showed fertilized prairies accrued more soil C and N than unfertilized prairies and corn systems, driven by increased microbial biomass, enzyme activity, and aggregation in fertilized prairie systems. Fertilized prairies had greater C-inputs in the form of roots than corn systems and greater inorganic N inputs, in the form of fertilizer, than unfertilized prairies. Thus, coupling of C and N inputs in fertilized prairies facilitated microbial growth and activity, which in turn enhanced soil aggregation, protection of microbially-processed organic matter, and total soil C and N pools. In summary, this dissertation provides a replicable method for soil aggregate isolation that can be used to test hypotheses about soil habitat influences on microbial communities and activities. It was determined that soil enzyme activity varied between aggregate fractions similarly in three managed ecosystems, reflecting consistent aggregate-level controls on enzyme activities. Fungal community structure was also influenced by aggregate fraction, but the ecosystem-level response was much greater. Together, differences in aggregate habitat, in concert with plant inputs and management regime, influenced C and N cycling in

corn, unfertilized and fertilized prairie ecosystems. Thus, knowledge of microbial responses at the soil aggregate scale can refine and improve scientific understanding of terrestrial ecosystem biogeochemistry.

CHAPTER I

GENERAL INTRODUCTION

The Role of Soil Ecology in Carbon and Nutrient Cycling

Soil stores approximately $1500 \cdot 10^{15}$ g carbon (C) globally, twice as much C as is found in the atmosphere and three times as much as is stored in terrestrial vegetation (Schlesinger, 1997). Similarly, soils store nutrients such as nitrogen (N) and phosphorus (P) which are critical to plant growth, including crop production, but can easily leach from soil and negatively impact surface waters (Renwick *et al.*, 2008). Soil organisms are the engines cycling C, N, and P through soils as they utilize organic matter, primarily plant root and litter inputs, for energy and nutrients (Falkowski *et al.*, 2008). Carbon and nutrient accrual and storage in soils is the result of the balance between microbial processing of organic matter and complete mineralization of organic matter to gasses including greenhouse gases carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4) (Schmidt *et al.*, 2011). Plant inputs, soil habitat, and management processes all influence soil microbial communities and their activities, driving the net balance of C and nutrient storage in and loss from soils.

Soil microorganisms decompose plant tissues and exudates by excreting enzymes into the environment to breakdown large plant macromolecules such as cellulose, hemicellulose, and lignin. Extracellular enzymes were first reported in soils well over a century ago (Woods 1899, as cited by (Skujins, 1978)), and many assays have been developed, enabling scientists to examine plant litter decomposition at the molecular

level (Sinsabaugh *et al.*, 1993). Break-down of plant macromolecules by extracellular enzymes is the first step in decomposition, and end-products may not be mineralized completely. Thus, extracellular enzyme activities provide insight into the microbial processing of plant-derived organic matter which contribute to soil organic matter pools with long residence time (Grandy & Neff, 2008).

Microbial production of extracellular enzymes is affected by both substrate availability and microbial community. Increased belowground allocation of C by plants enhances the abundance and activity of microorganisms, resulting in greater processing of C substrates and resulting soil organic matter (Angers & Caron, 1998; DeGrood *et al.*, 2005). Increased specific substrate availability stimulates cellulase and peptidase activities, but relative C and N availability limits enzyme activity at high substrate concentrations (Geisseler & Horwath, 2009). Soil microbes can respond to changes in plant inputs physiologically by changing their relative investments in extracellular enzymes (Hargreaves & Hofmockel, 2014; Stone *et al.*, 2014). Changes in extracellular enzyme activity can also be the consequence of shifts in microbial community membership, particularly fungi (Kaiser *et al.*, 2010). Microbial production of extracllular enzymes is only part of realized enzyme activity. Soil habitats constrain interactions between microbes, enzymes, and substrates; thus, examining microbes and enzyme activities within soil habitats is crucial to understanding C and N cycling within ecosystems.

Ecological interactions in soil are strongly influenced by soil structure, specifically the hierarchical distribution of soil aggregate fractions (Tisdall & Oades, 1982). Organic matter becomes physically enmeshed within larger aggregates, and

therefore protected from decomposition by soil organisms outside the aggregate (Six *et al.*, 2000a). Within large macroaggregates (>2000 μm), organic matter and soil particles fuse, forming microaggregates (<250 μm). When the large macroaggregate disintegrates, these microaggregates can persist in the soil matrix or become the nucleus for formation of new large macroaggregates (Six *et al.*, 2000a). Large macroaggregates naturally turn-over in soils, falling apart in response to a major rainfall event, freeze-thaw cycle, or force from an organism moving through the soil; aggregates re-form through physical and chemical forces from roots, fungi, earthworms, and bacteria (Tisdall, 1994; Jastrow, 1996; Angers & Caron, 1998). From a microbial perspective, the dynamics of soil aggregate turn-over not only result in dramatic changes in habitat, but also changes in accessible substrate. The interactions between soil aggregates and soil biology, including extracellular enzyme activity, have been an expanding area of scientific research in the past 30 years (Six *et al.*, 2004). Isolation and measurement of solid soil aggregates provides a useful means for separating unique zones of microbial colonization and activity (Mummey & Stahl, 2004; Bailey *et al.*, 2012b; Davinic *et al.*, 2012; Bailey *et al.*, 2013a; Bailey *et al.*, 2013b). Considering microbial communities and enzyme activities at the soil aggregate scale can give insight to ecological drivers of at the scale of the microbe and provide an approach to scale these micro-scale interactions to ecosystem-level changes in soil C and N cycling.

Through physical protection of organic matter, selection on microbial community structure, and ecological constraints on microbial physiology, soil aggregates influence ecosystem-level cycling of C, N, and P (Grandy & Neff, 2008; King, 2011). Coupling soil aggregate distribution with microbial activity is a promising approach to identifying

micro-scale factors that drive ecosystem scale decomposition. Integration of habitat influences on extracellular enzyme activities would enhance understanding of decomposition dynamics beyond resource limitations observed from integrating C, N, and P cycling extracellular enzyme activities at the ecosystem scale (Sinsabaugh *et al.*, 2008; Sinsabaugh *et al.*, 2009). Managed ecosystems provide unique opportunities in which to integrate soil physical and biological data because soil habitat and substrate resources are strongly manipulated. Furthermore, improved understanding of the ecological drivers of soil microbial communities and associated C and N cycling have important applications to management of agroecosystems to meet crop production goals and reduce negative environmental impacts.

Soil Carbon and Nitrogen Cycling in Bioenergy Production

Soils of central North America, including Iowa, are among the most fertile in the world (Eswaran *et al.*, 2003), and are the economic and ecological foundation of both rural and urban communities, throughout the region. Much of the region's productivity is related to the high levels of organic matter in its soils, derived from the growth and decay of native tallgrass prairie vegetation and charcoal produced by prairie fires (Buol *et al.*, 2003; Rodionov *et al.*, 2010). Nonetheless, despite their original endowment with an abundance of carbon (C), use of these soils for arable crop production since the mid-1800s has led to substantial reductions in C levels (Matson *et al.*, 1997; Stevenson & Cole, 1999).

In recent decades, there has been much research interest in rebuilding lost soil C stocks through agroecosystem management (Cambardella & Elliott, 1992; Cambardella & Elliott, 1994; Six *et al.*, 2000b; Six *et al.*, 2006; Russell *et al.*, 2009) and reconstruction of native grasslands (Baer *et al.*, 2002; Allison *et al.*, 2005; Matamala *et al.*, 2008; Bach *et al.*, 2010; Baer *et al.*, 2010; Bach *et al.*, 2012). Soil aggregate size and stability are reduced in cultivated systems compared with native grasslands (Cambardella & Elliott, 1992; Cambardella & Elliott, 1993; Six *et al.*, 1998). Native grasslands have greater soil organic matter content (McLauchlan, 2006) and microbial biomass (O'Donnell *et al.*, 2001). Conversion of cultivated lands into native grassland leads to improved soil structure (Jastrow *et al.*, 1998; Baer *et al.*, 2010)), increased soil C and N (Baer *et al.*, 2002; Matamala *et al.*, 2008; Baer *et al.*, 2010; O'Brien *et al.*, 2010), and larger, more diverse soil microbial communities (McKinley et al. 2005, Allison et al. 2005, Allison et al. 2007). In general grassland reconstruction leads to greater increases in soil C and internal cycling on N than conservation agroecosystem management, but provide almost no profitable product for landowners to sell into current agricultural commodity markets.

Recently, scientists have proposed utilizing native, reconstructed grasslands for cellulosic bioenergy feedstock (Tilman *et al.*, 2006). The U.S. Energy Independence and Security Act of 2007 mandated biofuel production to increase more than 7 fold over the next 15 years, and 68% of that increased production is required to come from non-cornstarch based biofuels (Congress, 2007). Use of native grasslands as cellulosic bioenergy feedstock could meet economic production goals and provide crucial ecological benefits (Tilman *et al.*, 2009). In fact, conversion of row-crop grain agroecosystems to perennial cellulose-based systems, including on marginal lands, could

serve as net sinks for greenhouse gasses (Robertson *et al.*, 2011; Gelfand *et al.*, 2013). However, increased demand for both corn grain and litter for bioenergy feedstock, could reduce extant grasslands, including those in conservation programs, as landowners shift those fields into corn production (Secchi *et al.*, 2008). Modeling has shown extensive use of grain crop residue for cellulosic biofuels may actually increase CO_2 emissions and decrease soil C stocks (Liska *et al.*, 2014). As such, scientific understand of the mechanisms driving the balance of C (and N) accrual and cycling in bioenergy systems is paramount to confronting these environmental challenges, yet there is limited empirical research focused on the ecological drivers of these microbial processes.

In this dissertation, I investigate ecological interactions at the microbial scale in soils to determine potential mechanisms driving ecosystem-level C and N cycling in reconstructed prairie and agroecosystems managed for bioenergy feedstock production. Chapter two presents a refined "optimal moisture" method for isolating soil aggregates to test biological hypotheses about community and functioning at the aggregate scale. Chapter three applies this method to measure microbial extracellular enzyme activity within soil aggregates sampled at nine dates across two years in three bioenergy cropping systems. This approached also enabled me to couple soil aggregate turnover and formation events with seasonal changes in C and N cycling enzyme activity. Chapter four utilizes the optimal moisture aggregate isolation method to investigate soil fungal community structure within soil aggregates and whole soil at two sampling dates within the three bioenergy cropping systems. Chapter five scales out from the aggregate-level and presents whole ecosystem C and N cycling in the three bioenergy systems. Together, these datasets provide novel understanding of extracellular enzyme activity and fungal

community structure at the aggregate-scale and provide a direct connection between aggregate-scale microbial ecology and ecosystem biogeochemistry.

Literature Cited

Allison, V. J., R. M. Miller, J. D. Jastrow, R. Matamala, and D. R. Zak. 2005. Changes in soil microbial community structure in a tallgrass prairie chronosequence. Soil Science Society of America Journal **69**:1412-1421.

Angers, D. A. and J. Caron. 1998. Plant-induced changes in soil structure: Processes and feedbacks. Biogeochemistry **42**:55-72.

Bach, E. M., S. G. Baer, C. K. Meyer, and J. Six. 2010. Soil texture affects soil microbial and structural recovery during grassland restoration. Soil Biology & Biochemistry **42**:2182-2191.

Bach, E. M., S. G. Baer, and J. Six. 2012. Plant and Soil Responses to High and Low Diversity Grassland Restoration Practices. Environmental Management **49**:412-424.

Baer, S. G., D. J. Kitchen, J. M. Blair, and C. W. Rice. 2002. Changes in ecosystem structure and function along a chronosequence of restored grasslands. Ecological Applications **12**:1688-1701.

Baer, S. G., C. K. Meyer, E. M. Bach, R. P. Klopf, and J. Six. 2010. Contrasting ecosystem recovery on two soil textures: implications for carbon mitigation and grassland conservation. Ecosphere **1**:art5.

Bailey, V. L., C. L. Bilskis, S. J. Fansler, L. A. McCue, J. L. Smith, and A. Konopka. 2012. Measurements of microbial community activities in individual soil macroaggregates. Soil Biology and Biochemistry **48**:192-195.

Bailey, V. L., S. J. Fansler, J. C. Stegen, and L. A. McCue. 2013a. Linking microbial community structure to [beta]-glucosidic function in soil aggregates. ISME J **7**:2044-2053.

Bailey, V. L., L. A. McCue, S. J. Fansler, M. I. Boyanov, F. DeCarlo, K. M. Kemner, and A. Konopka. 2013b. Micrometer-scale physical structure and microbial composition of soil macroaggregates. Soil Biology & Biochemistry **65**:60-68.

Buol, S. W., R. J. Southard, R. C. Graham, and P. A. McDaniel. 2003. Soil genesis and classification 5th edition. Iowa State University Pess - Blackwell, Ames, IA.

Cambardella, C. A. and E. T. Elliott. 1992. Particulate soil organic-matter changes across a grassland cultivation sequence. Soil Science Society of America Journal **56**:777-783.

Cambardella, C. A. and E. T. Elliott. 1993. Carbon and Nitrogen Distribution in Aggregates from Cultivated and Native Grassland Soils. Soil Science Society of America Journal **57**:1071-1076.

Cambardella, C. A. and E. T. Elliott. 1994. Carbon and nitrogen dynamics of soil organic-matter fractions from cultivated grassland soils. Soil Science Society of America Journal **58**:123-130.

Congress, U. t. 2007. Energy Independence and Security Act of 2007.*in* U. Congress, editor. Public Law 110-140.

Davinic, M., L. M. Fultz, V. Acosta-Martinez, F. J. Calderon, S. B. Cox, S. E. Dowd, V. G. Allen, J. C. Zak, and J. Moore-Kucera. 2012. Pyrosequencing and mid-infrared spectroscopy reveal distinct aggregate stratification of soil bacterial communities and organic matter composition. Soil Biology and Biochemistry **46**:63-72.

DeGrood, S. H., V. P. Claassen, and K. M. Scow. 2005. Microbial community composition on native and drastically disturbed serpentine soils. Soil Biology & Biochemistry **37**:1427-1435.

Eswaran, H., F. H. Beinroth, and P. F. Reich. 2003. A global assessment of land quality. Edward Elgar Publishing Ltd, Cheltenham.

Falkowski, P. G., T. Fenchel, and E. F. Delong. 2008. The microbial engines that drive Earth's biogeochemical cycles. Science **320**:1034-1039.

Geisseler, D. and W. R. Horwath. 2009. Relationship between carbon and nitrogen availability and extracellular enzyme activities in soil. Pedobiologia **53**:87-98.

Gelfand, I., R. Sahajpal, X. Zhang, R. C. Izaurralde, K. L. Gross, and G. P. Robertson. 2013. Sustainable bioenergy production from marginal lands in the US Midwest. Nature **493**:514-517.

Grandy, A. S. and J. C. Neff. 2008. Molecular C dynamics downstream: The biochemical decomposition sequence and its impact on soil organic matter structure and function. Science of the Total Environment **404**:297-307.

Hargreaves, S. K. and K. S. Hofmockel. 2014. Physiological shifts in the microbial community drive changes in enzyme activity in a perennial agroecosystem. Biogeochemistry **117**:67-79.

Jastrow, J. D. 1996. Soil aggregate formation and the accrual of particulate and mineral-associated organic matter. Soil Biology & Biochemistry **28**:665-676.

Jastrow, J. D., R. M. Miller, and J. Lussenhop. 1998. Contributions of interacting biological mechanisms to soil aggregate stabilization in restored prairie. Soil Biology & Biochemistry **30**:905-916.

Kaiser, C., M. Koranda, B. Kitzler, L. Fuchslueger, J. Schnecker, P. Schweiger, F. Rasche, S. Zechmeister-Boltenstern, A. Sessitsch, and A. Richter. 2010. Belowground carbon allocation by trees drives seasonal patterns of extracellular enzyme activities by altering microbial community composition in a beech forest soil. New Phytologist **187**:843-858.

King, G. M. 2011. Enhancing soil carbon storage for carbon remediation: potential contributions and constraints by microbes. Trends in Microbiology **19**:75-84.

Liska, A. J., H. Yang, M. Milner, S. Goddard, H. Blanco-Canqui, M. P. Pelton, X. X. Fang, H. Zhu, and A. E. Suyker. 2014. Biofuels from crop residue can reduce soil carbon and increase CO2 emissions. Nature Clim. Change **4**:398-401.

Matamala, R., J. D. Jastrow, R. M. Miller, and C. T. Garten. 2008. Temporal changes in C and N stocks of restored prairie: Implications for C sequestration strategies. Ecological Applications **18**:1470-1488.

Matson, P. A., W. J. Parton, A. G. Power, and M. J. Swift. 1997. Agricultural Intensification and Ecosystem Properties. Science **277**:504-509.

McLauchlan, K. 2006. The nature and longevity of agricultural impacts on soil carbon and nutrients: A review. Ecosystems **9**:1364-1382.

Mummey, D. L. and P. D. Stahl. 2004. Analysis of soil whole- and inner-microaggregate bacterial communities. Microbial Ecology **48**:41-50.

O'Brien, S. L., J. D. Jastrow, D. A. Grimley, and M. A. Gonzalez-Meler. 2010. Moisture and vegetation controls on decadal-scale accrual of soil organic carbon and total nitrogen in restored grasslands. Global Change Biology **16**:2573-2588.

O'Donnell, A. G., M. Seasman, A. Macrae, I. Waite, and J. T. Davies. 2001. Plants and fertilisers as drivers of change in microbial community structure and function in soils. Plant and Soil **232**:135-145.

Renwick, W. H., M. J. Vanni, Q. Zhang, and J. Patton. 2008. Water Quality Trends and Changing Agricultural Practices in a Midwest U.S. Watershed, 1994–2006. Journal of Environmental Quality **37**:1862-1874.

Robertson, G. P., S. K. Hamilton, S. J. Del Grosso, and W. J. Parton. 2011. The biogeochemistry of bioenergy landscapes: carbon, nitrogen, and water considerations. Ecological Applications **21**:1055-1067.

Rodionov, A., W. Amelung, N. Peinemann, L. Haumaier, X. D. Zhang, M. Kleber, B. Glaser, I. Urusevskaya, and W. Zech. 2010. Black carbon in grassland ecosystems of the world. Global Biogeochemical Cycles **24**.

Russell, A. E., C. A. Cambardella, D. A. Laird, D. B. Jaynes, and D. W. Meek. 2009. Nitrogen fertilizer effects on soil carbon balances in Midwestern US agricultural systems. Ecological Applications **19**:1102-1113.

Schlesinger, W. H. 1997. Biogeochemistry: An Analysis of Change. 2nd edition. Academic Press, San Diego, CA.

Schmidt, M. W. I., M. S. Torn, S. Abiven, T. Dittmar, G. Guggenberger, I. A. Janssens, M. Kleber, I. Kogel-Knabner, J. Lehmann, D. A. C. Manning, P. Nannipieri, D. P. Rasse, S. Weiner, and S. E. Trumbore. 2011. Persistence of soil organic matter as an ecosystem property. Nature **478**:49-56.

Secchi, S., J. Tyndall, L. A. Schulte, and H. Asbjornsen. 2008. High crop prices and conservation: Raising the Stakes. Journal of Soil and Water Conservation **63**:68A-73A.

Sinsabaugh, R. L., R. K. Antibus, A. E. Linkins, C. A. McClaugherty, L. Rayburn, D. Repert, and T. Weiland. 1993. Wood Decomposition: Nitrogen and Phosphorus Dynamics in Relation to Extracellular Enzyme Activity. Ecology **74**:1586-1593.

Sinsabaugh, R. L., B. H. Hill, and J. J. F. Shah. 2009. Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. Nature **462**:795-U117.

Sinsabaugh, R. L., C. L. Lauber, M. N. Weintraub, B. Ahmed, S. D. Allison, C. Crenshaw, A. R. Contosta, D. Cusack, S. Frey, M. E. Gallo, T. B. Gartner, S. E. Hobbie, K. Holland, B. L. Keeler, J. S. Powers, M. Stursova, C. Takacs-Vesbach, M. P. Waldrop, M. D. Wallenstein, D. R. Zak, and L. H. Zeglin. 2008. Stoichiometry of soil enzyme activity at global scale. Ecology Letters **11**:1252-1264.

Six, J., H. Bossuyt, S. Degryze, and K. Denef. 2004. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil & Tillage Research **79**:7-31.

Six, J., E. T. Elliott, and K. Paustian. 2000a. Soil macroaggregate turnover and microaggregate formation: A mechanism for C sequestration under no-tillage agriculture. Soil Biology & Biochemistry **32**:2099-2103.

Six, J., E. T. Elliott, K. Paustian, and J. W. Doran. 1998. Aggregation and soil organic matter accumulation in cultivated and native grassland soils. Soil Science Society of America Journal **62**:1367-1377.

Six, J., S. D. Frey, R. K. Thiet, and K. M. Batten. 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Science Society of America Journal **70**:555-569.

Six, J., K. Paustian, E. T. Elliott, and C. Combrink. 2000b. Soil structure and organic matter: I. Distribution of aggregate-size classes and aggregate-associated carbon. Soil Science Society of America Journal **64**:681-689.

Skujins, J. 1978. History of Abiontic Soil Enzyme Research. Pages 1-50 *in* R. G. Burns, editor. Soil Enzymes. Academic Press, London, UK.

Stevenson, F. J. and M. A. Cole. 1999. Cycles of soil : carbon, nitrogen, phosphorus, sulfur, micronutrients. John Wiley and Sons, Hoboken, NJ.

Stone, M. M., J. L. DeForest, and A. F. Plante. 2014. Changes in extracellular enzyme activity and microbial community structure with soil depth at the Luquillo Critical Zone Observatory. Soil Biology and Biochemistry **75**:237-247.

Tilman, D., J. Hill, and C. Lehman. 2006. Carbon-negative biofuels from low-input high-diversity grassland biomass. Science **314**:1598-1600.

Tilman, D., R. Socolow, J. A. Foley, J. Hill, E. Larson, L. Lynd, S. Pacala, J. Reilly, T. Searchinger, C. Somerville, and R. Williams. 2009. Beneficial Biofuels—The Food, Energy, and Environment Trilemma. Science **325**:270-271.

Tisdall, J. M. 1994. Possible Role of Soil-Microorganisms in Aggregation in Soils. Plant and Soil **159**:115-121.

Tisdall, J. M. and J. M. Oades. 1982. Organic-matter and water-stable aggregates in soils. Journal of Soil Science **33**:141-163.

CHAPTER II

SOIL AGGREGATE ISOLATION METHOD AFFECTS MEASURES OF INTRA-AGGREGATE EXTRACELLULAR ENZYME ACTIVITY

A paper published in *Soil Biology & Biochemistry* vol. 69: 54-62 (February 2014)

Elizabeth M. Bach and Kirsten S. Hofmockel

Abstract

Within soil aggregates, binding of organic matter is known to occlude it from microbial attack. Within aggregate fractions of different sizes, microbial communities and activities have also been shown to differ. As a result the soil physical structure, organic inputs and microbial activity together impact the rate at which organic matter is decomposed and stored within soil. However, methods developed for isolating soil aggregates may affect subsequent biological assays. In this study, we sought to understand how enzyme activity within soil aggregates is influenced by aggregate isolation methodology, including wet, dry, and "optimal moisture" sieving procedures within two contrasting ecosystems (corn agroecosystem and 2-yr old, diverse planted tallgrass prairie). Mass distribution of aggregates from wet-sieving was skewed toward small macroaggregates (250-1000 μm) and microaggregates (<250 μm), but the distribution of dry and optimal moisture aggregates was highly skewed toward large macroaggregates (>2000 μm). Wet-sieved macroaggregates (>1000 μm) had greater aggregate potential enzyme activity (nmol substrate h^{-1} g^{-1} dry aggregate) than smaller aggregate fractions and whole soil, particularly for C-cycling enzymes cellobiohydrolase and β-glucosidase. Also, wet-sieved aggregates from corn systems had higher potential

cellobiohydrolase and β-glucosidase activity than aggregates isolated from prairie. Neither of these relationships was observed in dry and optimal moisture aggregates, suggesting that elevated activities are characteristic of water-stable aggregates and possibly stimulated by soil rewetting. The proportional contribution to total enzyme activity observed in water-stable microaggregates accounted for 46-62% of whole soil activity; although water-stable large macroaggregates (>2000 μm) had greater aggregate enzyme activity, they contributed a minority of overall soil activity. In contrast, the proportional contribution of large macroaggregates comprised 70-78% of whole soil activity when dry sieved and 38-66% under optimal moisture sieving. Wet-sieving soil aggregates is most useful to examine long-term changes in soil organic matter and microbial activity between soil types. Optimal moisture and dry sieved aggregates may be useful alternatives to more closely capture short-term *in situ* measures of seasonal and intra-annual soil microbial activity.

Keywords: soil aggregates; extracellular enzyme activity; wet sieving; dry sieving; macroaggregate; microaggregates; water stable aggregates; optimal moisture

Introduction

Soil microbial ecologists face the difficult task of defining and quantifying microscopic habitats and relating ecological interactions in those micro-habitats to ecosystem level processes (Schimel & Schaeffer, 2012). Quantification of constraints on microbial activity within soil microhabitats is needed to better understand processing and stabilization of soil organic matter that result in storage of carbon (C) and nitrogen (N) in

soils or respiration of greenhouse gases (Schmidt *et al.*, 2011). Soil physical structure, specifically soil aggregation, is emerging as a critical factor influencing soil microbial communities and activities e.g. (Petersen *et al.*, 1997; Schutter & Dick, 2002; Allison & Jastrow, 2006; Ndour *et al.*, 2008; Briar *et al.*, 2011; Davinic *et al.*, 2012; Bailey *et al.*, 2013b). Soil aggregates, and more specifically intra-aggregate pore spaces, are the habitats in which microbes live and the physical constraints on microbial colonization, substrate availability, water and gas movement through these pores determines the environment in which microbes perform biogeochemical reactions that drive ecosystem functioning.

The most widely used traditional approach for soil aggregate isolation is wet-sieving. Wet-sieving aggregates involves submerging air dried soils in deionized water (slaking) and sieving by hand or machine through a series of sieves (Yoder, 1936; Elliott, 1986) (Fig. 2.1). Slaking soils generates a pressure gradient in which entrapped air causes disintegration of all but the most structurally stable aggregates (LeBissonnais, 1996; Saygin *et al.*, 2012). Soil scientists adopted the practice of slaking to represent soil surface disruption by rain and quantify potential erosion (Yoder, 1936; Elliott, 1986). However, the extent to which soils experience slaking forces *in situ* is not well understood. Wet-sieved aggregates are widely reported in the literature to differentiate soil structure and long-term changes in C-cycling pools between soils (Cambardella & Elliott, 1994; Jastrow, 1996; Six *et al.*, 2000a; Denef *et al.*, 2004; Wilson *et al.*, 2009; Chivenge *et al.*, 2011). Soil ecologists are also looking to soil aggregates as a means to detect differences in microbial communities and activities at a microbial scale. Wet-sieving has been a natural tool for isolating aggregates for biological analyses including

extracellular enzyme assays (Fansler *et al.*, 2005; Allison & Jastrow, 2006; Ndour *et al.*, 2008; Lagomarsino *et al.*, 2012), T-RFLP (Mummey & Stahl, 2004), PLFA (FAME) (Petersen *et al.*, 1997; Kong *et al.*, 2011), and pyrosequencing (Davinic *et al.*, 2012). These exciting and valuable studies have provided evidence that certain aggregate fractions support elevated microbial activity and potentially unique communities; however, more data is needed to relate observations at the aggregate scale to microbially-driven ecosystem processes.

Coupling measures of extracellular C-cycling enzyme activity within the same wet-sieved fractions can be an effective approach to investigating changes in microbial activity that drive long-term cycling of C (Fansler *et al.*, 2005; Marx *et al.*, 2005; Allison & Jastrow, 2006; Dorodnikov *et al.*, 2009). However, enzyme turnover times have been estimated on the order of hours to days (Allison, 2006). Mineral-stabilized enzymes may persist for months to years (Tabatabai & Dick, 2002), but mineral-stabilization may also inhibit the ability for enzyme active sites to bind with substrate (Allison, 2006). Additionally, it is well established that there is a pulse of microbial activity, releasing CO_2 when dry soils are rewetted (Stark & Firestone, 1995; Halverson *et al.*, 2000; Fierer & Schimel, 2003), coined the "Birch effect" (Birch & Friend, 1956). This flush of CO_2 has been detected within minutes of wetting in both the laboratory and field experiments (Borken *et al.*, 2003; Lee *et al.*, 2004; Sponseller, 2007) and is attributed to rapid microbial mineralization of soluble organic C compounds, end products of extracellular enzyme reactions. Thus, it is unclear how to interpret extracellular enzyme activity within wet-sieved aggregates in a broader ecosystem context.

Air-drying soil prior to slaking is important for determination of water-stable aggregate distribution as it maximizes the pressure gradient from air entrapped in aggregates to the surrounding water (Chenu & Cosentino, 2011). However, air-drying soils prior to sieving can impact measures of soil microbial communities and activities (Sparling & Cheshire, 1979; Wollum, 1994; Boone *et al.*, 1999). Extracellular enzyme activity and microbial biomass have been shown to decrease after air-drying soils, especially when field-moist soils are not in drought condition (Zornoza *et al.*, 2006; Zornoza *et al.*, 2007; Wallenius *et al.*, 2010; Peoples & Koide, 2012). In some studies, field moist soil is slaked directly, avoiding this additional complication (e.g. Allison & Jastrow, 2006; Ndour *et al.*, 2008; Davinic *et al.*, 2012). Field-moist soils with high moisture content will experience lower energy disruption during slaking because pores will already contain water, reducing the pressure gradient generated by slaking and leaving more macroaggregates intact. Aggregate-disrupting energy needs to be consistent to compare replicate samples within a study, and results across studies (Yoder, 1936; Panabokke & Quirk, 1957; LeBissonnais, 1996; LeBissonnais & Arrouays, 1997). Wet-sieving field moist samples can generate variable aggregate fraction distributions and result in heterogenous aggregate stability within isolated size fractions (Marquez *et al.*, 2004), which could obfuscate ecological patterns and impede interpretation of intra-aggregate biology at an ecosystem scale.

Since it is often difficult to perform biological assays immediately following aggregate isolation, aggregates are commonly frozen prior to analysis. Freezing field moist soils can affect extracellular enzyme activity (DeForest, 2009; Peoples & Koide, 2012), phospholipid fatty acids (Liu *et al.*, 2009; Wu *et al.*, 2009), and nucleic acids

(Lauber *et al.*, 2010; Rissanen *et al.*, 2010). Although effects of freezing field moist soils prior to biological measurements are well documented, the effects of freezing saturated soils, such as water-stable aggregates, are less understood. Flash-freezing saturated soils in liquid N has been shown to reduce denitrification enzyme activity (DEA) and nitrification enzyme activity (NEA) (Cooke, 1990). Freezing flooded paddy soils had no significant effect on total PLFA concentration, but did affect the distribution of PLFA types (Liu *et al.*, 2009). Freezing of water-saturated aggregates is a good example of novel challenges that can result from combining standard protocols from different disciplines.

A handful of studies have attempted to address the challenges that arise when performing biological analyses on aggregate fractions subjected to desiccation, rewetting, and/or saturation. Pioneered by Mendes *et al.* (1999) and coined the "optimal moisture" approach by Dorodnikov *et al.* (2009), this method involves gently breaking apart intact soil cores and allowing the soil to dry under controlled conditions (4°C) until soil reaches a consistent moisture to facilitate reproducible aggregate disruption (105-120 g water kg^{-1} soil, ~10-14% gravimetric water content) before being shaken to separate aggregates (Mendes *et al.*, 1999; Schutter & Dick, 2002; Muruganandam *et al.*, 2009; Bailey *et al.*, 2012a) (Fig. 2.1). These practices are designed to minimize impacts to the microbial community and biological parameters of interest and facilitate consistent energy in separation of aggregate fractions among sampling units.

In this study, we systematically investigated the effects of each step of the wet-sieving process on measured extracellular enzyme activity and related those measures back to whole soil measurements in two ecosystems. Our goal was to determine the best

approach for soil aggregate isolation to represent microbial-scale extracellular enzyme activity at the time of sampling for loamy soil under corn cultivation and planted tallgrass prairie. We utilized three aggregate isolation procedures to separate which parts of the physical disruption process were responsible for the greatest changes in extracellular enzyme activity: wet sieving, dry sieving, and optimal moisture sieving (Fig. 2.1). In order to focus on methodological effects, we used soils collected in May when both corn and prairie systems had minimal plant material inputs to minimize potential ecosystem interactions with activity. We hypothesized that 1) wet-sieving procedures would increase aggregate extracellular enzyme activity compared to dry and field-fresh whole soil because wetting results in release of soluble substrates and intracellular enzymes during the sieving process, 2) dry sieving procedures would reduce aggregate extracellular enzyme activity compared to field-fresh whole soil because of enzyme degradation during drying, and 3) optimal moisture sieving procedures would yield aggregate extracellular enzyme activity similar to field-fresh whole soil.

Materials & Methods

Study site

Soil was collected from the Iowa State University Comparison of Biofuel Systems (COBS) experimental site located on the South Reynoldson Farm in Boone County, IA (41°55'14.42"N, 93°44'58.96"W); see Jarchow and Liebman (2013) for a detailed site description. Soils consisted of loams in the Nicollet (Fine-loamy, mixed, superactive, mesic Aquic Hapludoll) and Webster (Fine-loamy, mixed, superactive, mesic Typic Endoaquoll) series with less than 3% slope. Sand content ranged from 27% to 53%

across the site and clay content ranged from 17% to 32%. In the 5 years prior to sampling, average growing season precipitation at the site was 91.8 cm and mean annual temperature was 8.9°C. The present study includes only two of the experimental agroecosystem treatments: no-till continuous corn (*Zea mays*) and planted tallgrass prairie (31 native species, sampled 2 years post-planting). Four replicate blocks contain one 27 m x 61 m plot of each planting treatment in a randomized complete block design (total n=8).

Soil Sampling

Soil for wet sieved aggregates and dry hand sieved aggregates was collected in May 2010 as part of another study (Wang *et al.*, 2012). Briefly, intact soil cores (7.5 cm diameter x 10 cm deep) were collected using a slide hammer soil core with inset aluminum rings from the continuous corn and prairie plots. Soil was transported back to the laboratory in the aluminum sleeves. Soils were broken along natural points of weakness and passed through a 10 mm sieve and allowed to air-dry completely. Gravimetric water content of the field moist soils was approximately 21% (Wang *et al.*, 2012). Sub-samples of whole soil were obtained from the sieved, field moist soil prior to drying and aggregate isolation by wet and dry sieving techniques.

In May 2011 additional soils were collected for analysis of "optimal moisture" sieved aggregates. Field conditions on May 2011 closely resembled field conditions in May 2010, and field-moist gravimetric water content averaged 19%. Soils were collected with a 10 cm diameter coring device to a depth of 10 cm, placed in plastic bags, stored at approximately 4°C, and transported to the laboratory. Three cores were collected from

each plot. Each core was gently broken up along natural points of weakness and passed through an 8 mm sieve, removing large roots and rocks. Each set of three cores was combined into one composite sample for each plot. Adapting the methods of Schutter and Dick (2002), soil was dried at 4°C in closed, sterilized plastic containers with desiccant until soil reached approximately 10% GWC (4 days). This was done to standardize soil moisture content prior to dry aggregate sieving and minimize microbial community changes during the drying period. Sub-samples of whole soil were obtained from the cold-dried soils prior to aggregate isolation.

Aggregate Fractionation

Soil aggregates were separated by three methods: wet sieving, dry sieving, and optimal moisture. A traditional wet sieving method was employed on dried soil collected in May 2010. Air-dry soil was placed on a stack of sieves including 4000 µm, 2000 µm, 1000 µm, and 250 µm mesh openings. The stack was agitated by machine in water at a rate of 90 strokes per minute for 5 minutes with a stroke length of approximately 2 cm. Sieves remained completely submerged in water for the entire stroke length (Wang *et al.*, 2012). Isolated wet aggregates were gently removed from the sieves and immediately frozen for use in potential enzyme assays. Aggregate and whole soil samples were frozen six months prior to enzyme analysis. The wet sieving method was repeated with a second sub-sample of dried soil to determine the distribution of wet aggregate fractions. As a follow-up to initial results, the effects of freezing saturated aggregates was tested on soils collected in May 2012, following the methods above, except that sub-samples of

aggregates were subjected to immediate analysis for extracellular enzyme activity or frozen for analysis three months later.

Dry aggregates were separated by hand placing air-dry soil collected in May 2010 on a stack of sieves, including 4000 µm, 2000 µm, 1000 µm, and 250 µm mesh openings. The stack was shaken horizontally, by hand at a rate of 30 times per minute for 2 minutes (Wang *et al.*, 2012). The resulting aggregate fractions were gently removed from the sieves and frozen immediately for use in soil enzyme assays. Aggregate and whole soil samples were frozen six months prior to enzyme analysis. The dry sieving method was repeated with a second sub-sample of dried soil to determine the distribution of aggregate fractions.

In May 2011, "optimal moisture" aggregates were isolated by utilizing a circular sieve shaker machine, modifying the procedure used by (Schutter & Dick, 2002). Approximately five hundred grams of soil was placed on a stack of sieves including 2000 µm, 1000 µm, and 250 µm mesh openings. The stack was bolted to a sieve shaker intended for soil particle analysis (CSI Scientific). The stack was shaken at approximately 200-250 rpms for three minutes. Soil was gently removed from each sieve and weighed to determine the distribution of aggregates. A sub-sample of each aggregate fraction was saved and immediately frozen for use in potential enzyme assays. Aggregate and whole soil samples were frozen for one month prior to enzyme analysis. Aggregates isolated from all methods are referred to by size: large macroaggregates (>2000 µm), medium macroaggregates (1000-2000 µm), small macroaggregates (250-1000 µm), and microaggregates (<250 µm).

Soil Enzyme Analysis

Extracellular soil enzyme assays were modified from Marx *et al.* (2001a) and DeForest (2009). Briefly, 1 g of frozen aggregates or whole soil was suspended in 250 mL sodium acetate buffer with the pH adjusted to the median of soils being tested to represent enzyme activity at field pH. Soils were pipetted into 96-well black micro-plates, with eight analytical replicates of each assay. Enzyme activities were determined by adding 4-Methylumbelliferyl (MUB)-linked substrates for N-cycling enzyme N-acetyl-glucosaminidase (NAG) and C-cycling enzymes β-glucosidase (BG), β-xylosidase (BX), and cellobiohydrolase (CB), for a final concentration of 40 μM. The plates also included blank columns including only buffer, negative controls including only buffer + substrate, reference standards including buffer + MUB, sample controls including soil + buffer, and "quench" controls including soil + MUB. Assays for NAG were incubated for 30 minutes, and BG, CB, and BX were incubated for 2 hours. After incubation, all reactions were stopped with 0.5 M NaOH and the solution optical density determined fluorometrically at 450 nm on a microplate reader (BioTek, Winooski, VT). For each aggregate fraction, aggregate potential enzyme activity, or the activity per gram of dry aggregate, was calculated and reported as nmol h^{-1} g^{-1} dry aggregate according to (DeForest, 2009; German *et al.*, 2012a). Potential extracellular enzyme activity was also expressed as a proportion of whole soil activity as follows:

$$\text{EEA}(\text{nmol } h^{-1} g^{-1} \text{ dry aggregate}) * \text{P}(\text{aggregate fraction})$$

where EEA is extracellular enzyme activity per gram dry aggregate and P(aggregate fraction) is the proportion of whole soil mass comprised in aggregate fraction of interest (Fansler *et al.*, 2005).

Statistical Analysis

A modified split-plot ANOVA was used to compare potential enzyme activities between aggregate fractions and ecosystem from each isolation method independently , accounting for the non-independence of aggregate and plot (Briar *et al.*, 2011). Ecosystem (corn or prairie) was considered the main effect and aggregate fraction a sub-plot factor. Aggregate fraction, ecosystem, and their interaction were included as fixed effects. Block and the block by crop interaction were included as random effects. Means from aggregate fractions and ecosystems were compared using Tukey's honestly significant difference. Natural log transformations were performed to satisfy ANOVA assumptions as needed. Models were run using PROC MIXED in SAS v. 9.2 (SAS Institute, Cary, NC). The authors fully acknowledge the limitations of having samples obtained from varying years, and we conducted a 2-way ANOVA comparison of all whole soil samples to compare ecosystem and sampling date effects and found no differences. In order to address if/how the ecological interpretation of the data varied by isolation method, we compared the results of the ANOVA model run for each isolation method individually rather than a direct contrast of raw enzyme activity values obtained in each isolation method.

Results

Wet-sieving

Comparison of whole soil enzyme activity showed no difference in potential activity for 2010 and 2011, using 2-way ANOVA comparison of all whole soil ($P>0.9$), so we proceeded with parallel comparisons of the three aggregate isolation methods. The

distribution of water-stable aggregates was skewed toward small macroaggregates (250-100 μm) and microaggregates and did not differ between ecosystems (Fig. 2.2). Cellobiohydrolase, β-glucosidase, β-xylosidase, and N-acetyl-glucosaminidase activity within water-stable aggregate fractions was highly variable, but was greater in large macroaggregates (>2000 μm) than whole soil (Table 2.1). β-xylosidase and N-acetyl-glucosaminidase activity in macroaggregates was also greater than microaggregates (<250 μm) ($P<0.02$, Table 2.1). There was no significant interaction with ecosystem ($P>0.2$) and thus, only the main effects of ecosystem and aggregate fraction are presented. Averaged across aggregate fractions, aggregate cellobiohydrolase and β-glucosidase activity was twofold greater in corn systems than two-year old prairie systems, and threefold greater within corn large macroaggregate fractions (>2000 μm) than prairie ($P<0.005$) (Fig. 2.3, Table 2.4S). Activity of C-cycling enzymes cellobiohydrolase, β-glucosidase, and β-xylosidase in corn systems also exceeded two-year old prairie systems in whole soil analysis prior to wet sieving ($P<0.03$, Table 2.4S). Variability in aggregate enzyme activity was high in water stable aggregates, and the coefficient of variation and standard error were greater than dry and optimal moisture aggregates of the same size, although only statistically significant for cellobiohydrolase ($P<0.02$, Table 2.5S). Freezing saturated water-stable aggregates prior to extracellular enzyme analysis did not affect detected potential enzyme activity, and thus did not contribute to differences observed specific to water-stable aggregates ($P>0.3$ for all enzymes, Table 2.6S).

Although we observed dramatically greater aggregate potential enzyme activity in macroaggregates (>1000 μm) than microaggregates (<250 μm) among wet sieved

fractions (Table 2.1), macroaggregates contributed a minor proportion of whole soil activity (Fig. 2.2). Proportionally, water-stable microaggregates (<250 μm) contributed the most to whole soil β-glucosidase activity (P=0.01; Table 2.2). This trend was observed, but was not significant for cellobiohydrolase, β-xylosidase, or N-acetyl-glucosaminidase (Table 2.2). It is interesting to note that wet-sieving generally yielded a percent recovery (weighted proportional sum of activity/whole soil activity) in excess of 100%. In other words, wet-sieving overestimated potential enzyme rates as measured independently from whole soil. Percent recovery within wet-sieved aggregates ranged from 92% to 198% of whole soil activity (Table 2.3). Mean percent recovery exceeded percent recovery from the other sieving methods for cellobiohydrolase (P=0.02, Table 2.3).

Dry Sieving

A dry-sieving procedure was used to eliminate the effect of slaking soil on aggregate fraction distribution and subsequent enzyme analysis. The distribution of aggregate fractions was heavily skewed toward large macroaggregates (>2000 μm) (Fig. 2.2). In contrast to water-stable aggregates, aggregate potential extracellular enzyme activity did not differ among dry-sieved aggregate fractions or whole soil for any of the enzymes measured (Table 2.1). No ecosystem effect was detected within aggregate fractions (P>0.16 for all enzymes). However, as noted above, there was less variability in enzyme activity, as measured by coefficient of variation and standard error, within dry sieved aggregate fractions compared with water stable aggregates of the same size (Table 2.5S). As a result of the aggregate distribution, large macroaggregates (>2000 μm)

contributed the greatest proportion of whole soil activity for all enzymes ($P<0.0001$ for all; Table 2.2). The cumulative sum of proportional enzyme activity did not differ from whole soil activity for any enzyme (data not shown, $P\geq0.48$); percent recovery ranged from 70% to 130% across all enzymes (Table 2.3).

Optimal moisture

An optimal moisture sieving approach was used to eliminate the effect of air-drying soils for extended periods of time prior to sieving and subsequent enzyme analysis. Optimal moisture sieving produced an aggregate distribution heavily skewed toward large macroaggregates (>2000 μm, Fig. 2.2). In contrast to water-stable aggregates, aggregate β-glucosidase activity was lowest in large macroaggregates (>2000 mm) and highest in whole soil ($P=0.03$), but there was no effect of aggregate size on aggregate potential enzyme activity for cellobiohydrolase, β-xylosidase, or N-acetyl-glucosaminidase (Table 2.1). No ecosystem effect was observed among aggregate fractions ($P\geq0.18$) or whole soil samples ($P\geq0.16$). As noted, variability of aggregate enzyme activity within optimal moisture aggregate fractions was less than in water-stable aggregates (Table 2.5S). The skewed distribution of aggregate fractions resulted in the large macroaggregates (>2000 μm) contributing the greatest proportion of whole soil enzyme activity and microaggregates (<250 μm) the least for all enzymes ($P<0.0001$ for all; Table 2.2). Cumulative proportional enzyme activity in optimal moisture aggregate fractions did not differ from whole soil (data not shown, $P\geq0.11$), and percent recovery ranged from 72% to 109% across enzymes (Table 2.3).

Discussion

Measurement of soil biological communities and activities at the aggregate scale is an emerging area of research in soil ecology (Bailey *et al.*, 2012a; Davinic *et al.*, 2012; Bailey *et al.*, 2013b). With the combination of soil biology and soil physics, come challenges in integrating methods to address novel hypotheses being tested, and the goal of our study was to quantify how aggregate isolation methodology may influence ecological interpretations of extracellular enzyme activity. Our data show clear evidence that soil aggregate isolation method strongly affects both the aggregate extracellular enzyme activity and proportional scaling of these activities to whole soil enzyme activities. Results from our independently analyzed data sets exhibit contrasting observations of extracellular enzyme activity distribution among soil aggregate fractions.

Slaking is a relatively high energy disruption, and only the most stable macroaggregates remain intact (Coughlan *et al.*, 1973; Cambardella & Elliott, 1993; Chenu & Cosentino, 2011). In this study, the mass distribution of water-stable aggregates was skewed toward small macroaggregates (250-1000 μm) and microaggregates (<250 μm). Enzyme activity measured within the wet-sieved fractions can provide valuable information as to the distribution of mineral stabilized enzymes across water-stable aggregates and soil particles, but it can be difficult to relate these data to enzyme-substrate interactions *in situ* (Allison & Jastrow, 2006).

Optimal moisture sieving is intended to facilitate separation of soil aggregate units with minimal impact on microbial communities and activities, including enzyme-substrate associations (Schutter & Dick, 2002; Dorodnikov *et al.*, 2009; Bailey *et al.*, 2013b). However, none of these previous studies directly compared measurements from

optimal moisture aggregates with wet- or dry-sieved aggregates. In our study optimal moisture and dry-sieved aggregates exhibited similar size distributions and potential enzyme activities, contrasting the results from wet-sieving. The distribution of dry and optimal moisture aggregates was highly skewed toward large macroaggregates (>2000 μm) due to the weaker lateral disruptive forces applied during sieving (Schutter & Dick, 2002; Dorodnikov *et al.*, 2009). Aggregate enzyme activity was similar across all fractions. Proportionally, macroaggregates comprised >60% of total soil activity, reflecting the aggregate size fraction distribution, suggesting the majority of total soil organic matter processes are occurring within macroaggregates that are not water stable.

The greatest observed difference between the three sieving methods was the four fold greater aggregate potential enzyme activity within wet-sieved macroaggregates compared with dry and optimal moisture macroaggregates (>2000 μm). When adjusted for the proportion of total soil each wet-sieved size fraction contributed, total potential enzyme activity exceeded 100% of activity in fresh whole soil, indicating additional enzyme activity was generated during the wet-sieving process. Several factors may stimulate extracellular enzyme activity resulting in laboratory-induced biological responses during the wet-sieving process. We tested the effect of freezing wet-sieved aggregates on potential enzyme activity and found no effect (Table 2.6S); thus, dry- and optimal moisture sieved aggregates were not subjected to a test of freezing effect. Length of storage time has been shown to affect extracellular enzyme activity (DeForest, 2009), and we acknowledge our samples were stored at -20°C for varying lengths of time, but the direction and magnitude of change in extracellular enzyme activities reported by DeForest (2009) was inconsistent across lengths of storage time and types of soil, and

mostly not statistically significant. All our samples, excepting fresh-analyzed wet-sieved aggregates, were stored in the freezer for more than one month and observed differences between sieving methods were at least an order of magnitude greater than the differences reported by DeForest (2009) resulting from storage time. Thus, differences between methods, specifically optimal moisture and wet-sieved aggregates likely reflect changes incurred in the aggregate sieving process, not during an additional five months of storage at -20°C.

Numerous biological factors may be driving the increase in extracellular enzyme activity in wet-sieved soil aggregates. Respired C from rewetted soils has been shown to originate from microbes, including intracellular molecules, dead microbial biomass, and/or extracellular polymeric substances (EPS) (Halverson *et al.*, 2000; Fierer & Schimel, 2003). Mucilages of microbial and plant origin are known to increase macroaggregate stability (Martin, 1946; Whistler & Kirby, 1956; Angers & Caron, 1998; Jastrow *et al.*, 1998; Rillig & Mummey, 2006) and wet-dry cycles have been shown to stimulate extracellular enzyme activity within EPS layers (Kemmling *et al.*, 2004). Another potential source of elevated cellobiohydrolase and β-glucosidase activity may be contact between microorganisms and soluble C compounds released during slaking. Bacteria can respond metabolically to changes in moisture in less than 15 minutes (Halverson *et al.*, 2000), and the wet-sieving process requires ~30 minutes to complete. Previous work has also found initiation of fungal conidia, or spore, germination within 10-30 minutes of contact with aqueous nutritive solutions, resulting in translation and assembly of cellulases from pre-packaged mRNA (Lamarre *et al.*, 2008; Metz *et al.*, 2011).

In addition to overall elevated activity, water-stable aggregates exhibited an ecosystem effect on extracellular enzyme activity that was not observed in dry and optimal moisture aggregates. The aforementioned microbial slaking responses may be operating differentially in the corn and prairie systems, resulting in a three-fold increase in aggregate enzyme activity within slaked large macroaggregates in corn systems compared with prairie. The lack of corroboration of the ecosystem response from water stable aggregates in dry and optimal moisture aggregates indicates the effect results from the rewetting process rather than soil physical constraints on extracellular enzyme activity. Wet sieving was originally developed to test the strength of aggregates against slaking and has been successfully applied to quantifying pools of C protected from turnover during slaking events (Elliott, 1986; Cambardella & Elliott, 1993; Six *et al.*, 2000b). However, soil microorganisms and associated activities are sensitive to wetting events. As such, water-stable aggregates may provide useful information in understanding soil microbial responses to rapid wetting events, but the optimal moisture approach may be better suited to interpreting *in situ* biological responses from aggregates sustained beyond a rewetting event.

Dry sieved and optimal moisture macroaggregates include both water-stable macroaggregates and macroaggregates that are not water-stable. These "non-stable aggregates," or "dry" aggregates, likely turnover rapidly in response to natural disturbance events such as rain, freeze, thaw, and dry-down (Yang & Wander, 1998; Kristiansen *et al.*, 2006; Yoo *et al.*, 2011; Wang *et al.*, 2012) as well as management practices such as tillage (Six *et al.*, 1998; Yang & Wander, 1998; Plante *et al.*, 2002), facilitating both mineralization of previously occluded organic matter and incorporation

of organic inputs into newly formed aggregates. Optimal moisture sieving can be used to determine the ecological context in which enzymes are interacting with substrate (Mendes *et al.*, 1999; Schutter & Dick, 2002; Dorodnikov *et al.*, 2009). Bailey *et al.* (2012a) used a similar optimal moisture approach to observe patchy distribution of microbial communities within aggregates with differing general activity levels and specific substrate decomposition capacity. We believe that optimal moisture sieving can be used in successive sampling to estimate turnover of "non-stable" aggregates and enzymatic responses to seasonal changes in substrate availability and inputs. Turnover of "non-stable" aggregates play an important role in C cycling and storage in soils because aggregate turnover balances occlusion and microbial processing of organic matter, which may increase chemical stability of organic matter as microbes mineralize simple polysaccharides leaving behind a greater proportion of complex organic molecules (Plante & McGill, 2002; Grandy & Neff, 2008; Yoo *et al.*, 2011). Aggregate distributions from periodic samplings (e.g. once a month) can be used to track changes in the proportion of macroaggregates as they breakdown and reform throughout the growing season (Yang & Wander, 1998).

Coupling biological measures such as extracellular enzyme activity and organic matter chemistry could provide insight into microbial processing and stabilization of C inputs within aggregate fractions. It should be noted that although we observed no difference in extracellular enzyme activity within dry and optimal moisture aggregates, the process of air-drying soils does change aggregate stability (Panabokke & Quirk, 1957; Francis & Cruse, 1983; Marquez *et al.*, 2004; Wang *et al.*, 2012) and microbial growth and activity (Roberson & Firestone, 1992; Zornoza *et al.*, 2007). The optimal

moisture approach may provide an advantage of minimizing microbial responses to lab processing for a wide-range of biological assays, enabling the testing of ecological hypotheses linking *in situ* microbial communities and activities with ecological processes.

Soil microbial ecology at the soil aggregate scale is an emerging area of research, and there is much to be learned by leveraging soil physicochemical methods to elucidate soil biology at the microbial scale. However, care must be taken when selecting methods most appropriate to integrate soil aggregates and biological assays. The goal of this study was to empirically evaluate three approaches to isolating soil aggregates to represent microbial-scale extracellular enzyme activity. Our first hypothesis was supported in that we found elevated aggregate enzyme activity within macroaggregates isolated by wet-sieving. Dry sieving did not reduce measured aggregate extracellular enzyme activity compared to field-fresh whole-soil as predicted. Optimal moisture sieving did yield extracellular enzyme activities similar to whole soil measurements. Increased microbial activity in rewetted soils is a commonly observed phenomenon, but has rarely been considered as contributing to differences observed in biological activity within water stable aggregate fractions. Slaking-induced differences in enzymatic activity have further ramifications for scaling-up to ecosystem-level processes, as indicated by our finding that proportional contribution of water stable macroaggregates to whole soil enzyme activity was <25%, but dry and optimal moisture large macroaggregates comprised >60% of whole soil and proportionally contributed the majority of whole soil enzyme activity. In conclusion, we recommend future work relating microbial communities and activities within soil aggregate fractions to ecosystem processes should carefully select aggregate

isolation methods which will minimize laboratory-induced responses and directly address the ecological hypothesis being tested.

Acknowledgements

The authors wish to acknowledge Enhang Wang, Eric Asbe, Megan Bartholomew, Austin Putz, Dade McBrayer, and Giselle Navarez for their assistance in the field and laboratory. Dr. Cynthia Cambardella, Sarah Hargreaves, Alison King, and Ryan Williams provided valuable feedback on drafts of this manuscript. Funding was provided by USDA-NASA grant 20116700330364.

Literature Cited

Allison, S.D., 2006. Soil minerals and humic acids alter enzyme stability: implications for ecosystem processes. Biogeochemistry 81, 361-373.

Allison, S.D., Jastrow, J.D., 2006. Activities of extracellular enzymes in physically isolated fractions of restored grassland soils. Soil Biology & Biochemistry 38, 3245-3256.

Angers, D.A., Caron, J., 1998. Plant-induced changes in soil structure: Processes and feedbacks. Biogeochemistry 42, 55-72.

Bailey, V.L., Bilskis, C.L., Fansler, S.J., McCue, L.A., Smith, J.L., Konopka, A., 2012. Measurements of microbial community activities in individual soil macroaggregates. Soil Biology & Biochemistry 48, 192-195.

Bailey, V.L., McCue, L.A., Fansler, S.J., Boyanov, M.I., DeCarlo, F., Kemner, K.M., Konopka, A., 2013. Micrometer-scale physical structure and microbial composition of soil macroaggregates. Soil Biology & Biochemistry 65, 60-68.

Birch, H.F., Friend, M.T., 1956. The organic-matter and nitrogen status of east African soils. Journal of Soil Science 7, 156-167.

Boone, R.D., Grigal, D.F., Sollins, D.F., Ahrens, R.J., Armstrong, D.E., 1999. Soil sampling, preparation, archiving, and quality control, In: Robertson, G.P., Coleman, D.C., Bledsoe, C.S., Sollins, P. (Eds.), Standard Soil Methods for Long-Term Ecological Research. Oxford University Press, New York, NY, pp. 3-28.

Borken, W., Davidson, E.A., Savage, K., Gaudinski, J., Trumbore, S.E., 2003. Drying and wetting effects on carbon dioxide release from organic horizons. Soil Science Society of America Journal 67, 1888-1896.

Briar, S.S., Fonte, S.J., Park, I., Six, J., Scow, K., Ferris, H., 2011. The distribution of nematodes and soil microbial communities across soil aggregate fractions and farm management systems. Soil Biology & Biochemistry 43, 905-914.

Cambardella, C.A., Elliott, E.T., 1993. Carbon and nitrogen distribution in aggregates from cultivated and native grassland soils. Soil Science Society of America Journal 57, 1071-1076.

Cambardella, C.A., Elliott, E.T., 1994. Carbon and nitrogen dynamics of soil organic-matter fractions from cultivated grassland soils. Soil Science Society of America Journal 58, 123-130.

Chenu, C., Cosentino, D., 2011. Microbial regulation of soil structural dynamics, In: Ritz, K., Young, I. (Eds.), The Architecture and Biology of Soils: Life in Inner Space. CABI, Oxfordshire, Oxford U.K., pp. 37-70.

Chivenge, P., Vanlauwe, B., Gentile, R., Six, J., 2011. Organic resource quality influences short-term aggregate dynamics and soil organic carbon and nitrogen accumulation. Soil Biology & Biochemistry 43, 657-666.

Cooke, J.G., 1990. Rapid freezing effects on nitrification and denitrification enzyme activity in saturated soil and aquatic sediments. Soil Biology & Biochemistry 22, 1171-1172.

Coughlan, K.J., Fox, W.E., Hughes, J.D., 1973. Aggregation in swelling clay soils. Australian Journal of Soil Research 11, 133-141.

Davinic, M., Fultz, L.M., Acosta-Martinez, V., Calderon, F.J., Cox, S.B., Dowd, S.E., Allen, V.G., Zak, J.C., Moore-Kucera, J., 2012. Pyrosequencing and mid-infrared spectroscopy reveal distinct aggregate stratification of soil bacterial communities and organic matter composition. Soil Biology and Biochemistry 46, 63-72.

DeForest, J.L., 2009. The influence of time, storage temperature, and substrate age on potential soil enzyme activity in acidic forest soils using MUB-linked substrates and L-DOPA. Soil Biology & Biochemistry 41, 1180-1186.

Denef, K., Six, J., Merckx, R., Paustian, K., 2004. Carbon sequestration in microaggregates of no-tillage soils with different clay mineralogy. Soil Science Society of America Journal 68, 1935-1944.

Dorodnikov, M., Blagodatskaya, E., Blagodatsky, S., Marhan, S., Fangmeier, A., Kuzyakov, Y., 2009. Stimulation of microbial extracellular enzyme activities by elevated CO2 depends on soil aggregate size. Global Change Biology 15, 1603-1614.

Elliott, E.T., 1986. Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. Soil Science Society of America Journal 50, 627-633.

Fansler, S.J., Smith, J.L., Bolton, H., Bailey, V.L., 2005. Distribution of two C cycle enzymes in soil aggregates of a prairie chronosequence. Biology and Fertility of Soils 42, 17-23.

Fierer, N., Schimel, J.P., 2003. A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. Soil Science Society of America Journal 67, 798-805.

Francis, P.B., Cruse, R.M., 1983. Soil-water matric potential effects on aggregate stability. Soil Science Society of America Journal 47, 578-581.

German, D.P., Weintraub, M.N., Grandy, A.S., Lauber, C.L., Rinkes, Z.L., Allison, S.D., 2012. Corrigendum to "Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies" [Soil Biol. Biochem. 43 (2011) 1387–1397]. Soil Biology & Biochemistry 44, 151.

Grandy, A.S., Neff, J.C., 2008. Molecular C dynamics downstream: The biochemical decomposition sequence and its impact on soil organic matter structure and function. Science of the Total Environment 404, 297-307.

Halverson, L.J., Jones, T.M., Firestone, M.K., 2000. Release of intracellular solutes by four soil bacteria exposed to dilution stress. Soil Science Society of America Journal 64, 1630-1637.

Jarchow, M.E., Liebman, M., 2013. Nitrogen fertilization increases diversity and productivity of prairie communities used for bioenergy. GCB Bioenergy 5, 281-289.

Jastrow, J.D., 1996. Soil aggregate formation and the accrual of particulate and mineral-associated organic matter. Soil Biology & Biochemistry 28, 665-676.

Jastrow, J.D., Miller, R.M., Lussenhop, J., 1998. Contributions of interacting biological mechanisms to soil aggregate stabilization in restored prairie. Soil Biology & Biochemistry 30, 905-916.

Kemmling, A., Kämper, M., Flies, C., Schieweck, O., Hoppert, M., 2004. Biofilms and extracellular matrices on geomaterials. Environmental Geology 46, 429-435.

Kong, A.Y.Y., Scow, K.M., Cordova-Kreylos, A.L., Holmes, W.E., Six, J., 2011. Microbial community composition and carbon cycling within soil microenvironments of conventional, low-input, and organic cropping systems. Soil Biology & Biochemistry 43, 20-30.

Kristiansen, S.M., Schjønning, P., Thomsen, I.K., Olesen, J.E., Kristensen, K., Christensen, B.T., 2006. Similarity of differently sized macro-aggregates in arable soils of different texture. Geoderma 137, 147-154.

Lagomarsino, A., Grego, S., Kandeler, E., 2012. Soil organic carbon distribution drives microbial activity and functional diversity in particle and aggregate-size fractions. Pedobiologia 55, 101-110.

Lamarre, C., Sokol, S., Debeaupuis, J.-P., Henry, C., Lacroix, C., Glaser, P., Coppee, J.-Y., Francois, J.-M., Latge, J.-P., 2008. Transcriptomic analysis of the exit from dormancy of Aspergillus fumigatus conidia. Bmc Genomics 9.

Lauber, C.L., Zhou, N., Gordon, J.I., Knight, R., Fierer, N., 2010. Effect of storage conditions on the assessment of bacterial community structure in soil and human-associated samples. Fems Microbiology Letters 307, 80-86.

LeBissonnais, Y., 1996. Aggregate stability and assessment of soil crustability and erodibility .1. Theory and methodology. European Journal of Soil Science 47, 425-437.

LeBissonnais, Y., Arrouays, D., 1997. Aggregate stability and assessment of soil crustability and erodibility .2. Application to humic loamy soils with various organic carbon contents. European Journal of Soil Science 48, 39-48.

Lee, X., Wu, H.-J., Sigler, J., Oishi, C., Siccama, T., 2004. Rapid and transient response of soil respiration to rain. Global Change Biology 10, 1017-1026.

Liu, Y., Yao, H., Huang, C., 2009. Assessing the effect of air-drying and storage on microbial biomass and community structure in paddy soils. Plant and Soil 317, 213-221.

Marquez, C.O., Garcia, V.J., Cambardella, C.A., Schultz, R.C., Isenhart, T.M., 2004. Aggregate-size stability distribution and soil stability. Soil Science Society of America Journal 68, 725-735.

Martin, J.P., 1946. Microorganisms and soil aggregation.2. Influence of bacterial polysaccharides on soil structure. Soil Science 61, 157-166.

Marx, M.C., Kandeler, E., Wood, M., Wermbter, N., Jarvis, S.C., 2005. Exploring the enzymatic landscape: distribution and kinetics of hydrolytic enzymes in soil particle-size fractions. Soil Biology & Biochemistry 37, 35-48.

Marx, M.C., Wood, M., Jarvis, S.C., 2001. A microplate fluorimetric assay for the study of enzyme diversity in soils. Soil Biology & Biochemistry 33, 1633-1640.

Mendes, I.C., Bandick, A.K., Dick, R.P., Bottomley, P.J., 1999. Microbial biomass and activities in soil aggregates affected by winter cover crops. Soil Science Society of America Journal 63, 873-881.

Metz, B., Seidl-Seiboth, V., Haarmann, T., Kopchinskiy, A., Lorenz, P., Seiboth, B., Kubicek, C.P., 2011. Expression of biomass-degrading enzymes is a major event during conidium development in *Trichoderma reesei*. Eukaryotic Cell 10, 1527-1535.

Mummey, D.L., Stahl, P.D., 2004. Analysis of soil whole- and inner-microaggregate bacterial communities. Microbial Ecology 48, 41-50.

Muruganandam, S., Israel, D.W., Robarge, W.P., 2009. Activities of nitrogen-mineralization enzymes associated with soil aggregate size fractions of three tillage systems. Soil Science Society of America Journal 73, 751-759.

Ndour, N.Y.B., Achouak, W., Christen, R., Heulin, T., Brauman, A., Chotte, J.-L., 2008. Characteristics of microbial habitats in a tropical soil subject to different fallow management. Applied Soil Ecology 38, 51-61.

Panabokke, C.R., Quirk, J.P., 1957. Effect of initial water content on stability of soil aggregates in water. Soil Science 83, 185-195.

Peoples, M.S., Koide, R.T., 2012. Considerations in the storage of soil samples for enzyme activity analysis. Applied Soil Ecology 62, 98-102.

Petersen, S.O., Debosz, K., Schjonning, P., Christensen, B.T., Elmholt, S., 1997. Phospholipid fatty acid profiles and C availability in wet-stable macro-aggregates from conventionally and organically farmed soils. Geoderma 78, 181-196.

Plante, A.F., Feng, Y., McGill, W.B., 2002. A modeling approach to quantifying soil macroaggregate dynamics. Canadian Journal of Soil Science 82, 181-190.

Plante, A.F., McGill, W.B., 2002. Intraseasonal soil macroaggregate dynamics in two contrasting field soils using labeled tracer spheres Soil Science Society of America Journal 66, 1285-1295.

Rillig, M.C., Mummey, D.L., 2006. Mycorrhizas and soil structure. New Phytologist 171, 41-53.

Rissanen, A.J., Kurhela, E., Aho, T., Oittinen, T., Tiirola, M., 2010. Storage of environmental samples for guaranteeing nucleic acid yields for molecular microbiological studies. Applied Microbiology and Biotechnology 88, 977-984.

Roberson, E.B., Firestone, M.K., 1992. Relationship between desiccation and exopolysaccharide production in a soil Pseudomonas sp. Applied and Environmental Microbiology 58, 1284-1291.

Saygin, S.D., Cornelis, W.M., Erpul, G., Gabriels, D., 2012. Comparison of different aggregate stability approaches for loamy sand soils. Applied Soil Ecology 54, 1-6.

Schimel, J., Schaeffer, S.M., 2012. Microbial control over carbon cycling in soil. Frontiers in Microbiology 3.

Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kogel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D.P., Weiner, S., Trumbore, S.E., 2011. Persistence of soil organic matter as an ecosystem property. Nature 478, 49-56.

Schutter, M.E., Dick, R.P., 2002. Microbial community profiles and activities among aggregates of winter fallow and cover-cropped soil. Soil Science Society of America Journal 66, 142-153.

Seech, A.G., Beauchamp, E.G., 1988. Denitrificaiton in soil aggregates of different sizes. Soil Science Society of America Journal 52, 1616-1621.

Six, J., Elliott, E.T., Paustian, K., 2000a. Soil macroaggregate turnover and microaggregate formation: A mechanism for C sequestration under no-tillage agriculture. Soil Biology & Biochemistry 32, 2099-2103.

Six, J., Elliott, E.T., Paustian, K., Doran, J.W., 1998. Aggregation and soil organic matter accumulation in cultivated and native grassland soils. Soil Science Society of America Journal 62, 1367-1377.

Six, J., Paustian, K., Elliott, E.T., Combrink, C., 2000b. Soil structure and organic matter: I. Distribution of aggregate-size classes and aggregate-associated carbon. Soil Science Society of America Journal 64, 681-689.

Sparling, G.P., Cheshire, M.V., 1979. Effects of soil drying and storage on subsequent microbial-growth. Soil Biology & Biochemistry 11, 317-319.

Sponseller, R.A., 2007. Precipitation pulses and soil CO2 flux in a Sonoran Desert ecosystem. Global Change Biology 13, 426-436.

Stark, J.M., Firestone, M.K., 1995. Mechanisms for soil-moisture effects on activity of nitrifying bacteria. Applied and Environmental Microbiology 61, 218-221.

Tabatabai, M.A., Dick, W.A., 2002. Enzymes in soil, In: Burns, R.G., Dick, R.P. (Eds.), Enzymes in the Environment. Marcel Dekker, Inc, New York, NY, pp. 567-596.

Wallenius, K., Rita, H., Simpanen, S., Mikkonen, A., Niemi, R.M., 2010. Sample storage for soil enzyme activity and bacterial community profiles. Journal of Microbiological Methods 81, 48-55.

Wang, E., Cruse, R.M., Chen, X., Daigh, A., 2012. Effects of moisture condition and freeze/thaw cycles on surface soil aggregate size distribution and stability. Canadian Journal of Soil Science 92, 529-536.

Whistler, R.L., Kirby, K.W., 1956. Composition and Behavior of Soil Polysaccharides1,2. Journal of the American Chemical Society 78, 1755-1759.

Wilson, G.W.T., Rice, C.W., Rillig, M.C., Springer, A., Hartnett, D.C., 2009. Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: Results from long-term field experiments. Ecology Letters 12, 452-461.

Wollum, A.G., 1994. Soil sampling for microbilogical analysis, In: Weaver, R.W., Angle, J.S., Bottomley, P.S. (Eds.), Methods of Soil Analysis: Part 2- Microbiological and Biochemical Properties. Soil Science Society of America, Madison, WI, pp. 1-14.

Wu, Y., Ding, N., Wang, G., Xu, J., Wu, J., Brookes, P.C., 2009. Effects of different soil weights, storage times and extraction methods on soil phospholipid fatty acid analyses. Geoderma 150, 171-178.

Yang, X.-M., Wander, M.M., 1998. Temporal changes in dry aggregate size and stability: tillage and crop effects on a silty loam Mollisol in Illinois. Soil and Tillage Research 49, 173-183.

Yoder, R.E., 1936. A direct method of aggregate analysis of soils and a study of the physical nature of erosion losses. Journal of the American Society of Agronomy 28, 337-351.

Yoo, G., Yang, X., Wander, M.M., 2011. Influence of soil aggregation on SOC sequestration: A preliminary model of SOC protection by aggregate dynamics. Ecological Engineering 37, 487-495.

Zornoza, R., Guerrero, C., Mataix-Solera, J., Arcenegui, V., García-Orenes, F., Mataix-Beneyto, J., 2006. Assessing air-drying and rewetting pre-treatment effect on some soil enzyme activities under Mediterranean conditions. Soil Biology & Biochemistry 38, 2125-2134.

Zornoza, R., Guerrero, C., Mataix-Solera, J., Arcenegui, V., García-Orenes, F., Mataix-Beneyto, J., 2007. Assessing the effects of air-drying and rewetting pre-treatment on soil microbial biomass, basal respiration, metabolic quotient and soluble carbon under Mediterranean conditions. European Journal of Soil Biology 43, 120-129.

Figures

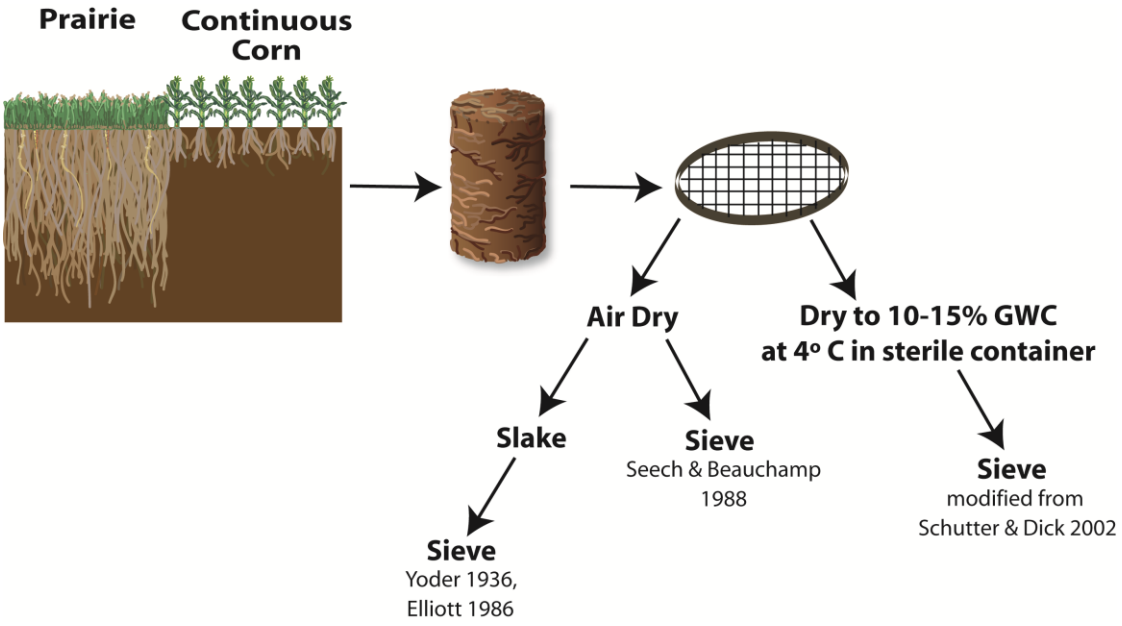

Figure 2.1: Schematic flow of aggregate sieving methods and pre-treatments. Field soil was collected as an intact core, gently passed through 8mm sieve and either air-dried in preparation for dry and wet sieving or partially dried at 4°C in sterile containers for optimal moisture sieving.

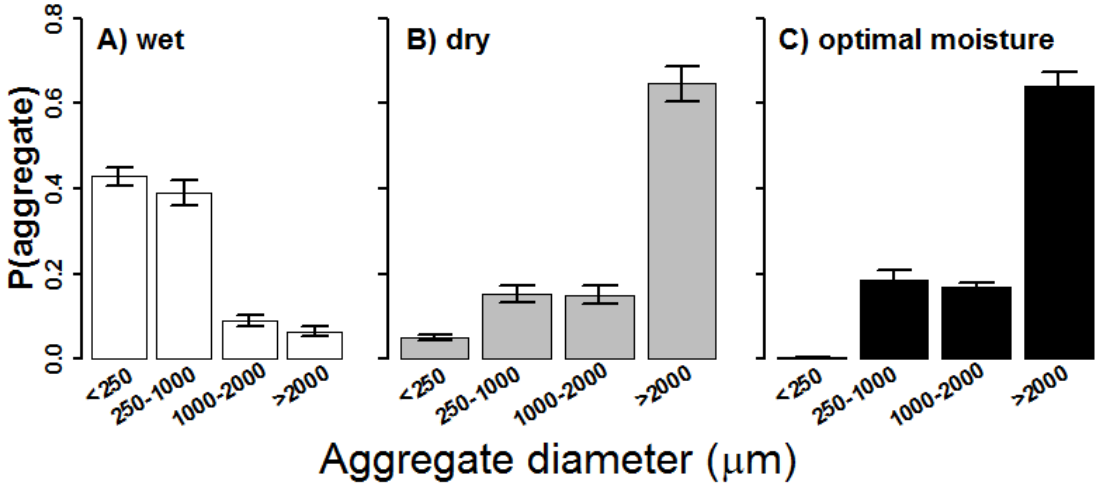

Figure 2.2: Proportion of soil aggregate fractions (± 1 SE) isolated through wet (A), dry (B), and optimal moisture(C) sieving procedures. Data are averaged across ecosystem type. Microaggregates and small macroaggregates comprised the greatest proportion of whole soil mass with wet-sieving and were significantly greater than dry or optimal moisture fractions of the same size ($P<0.001$). Dry and optimal moisture macroaggregates >2000 μm comprised the greatest proportion of whole soil mass and were significantly greater than wet sieved macroaggregates >2000 μm ($P<0.001$).

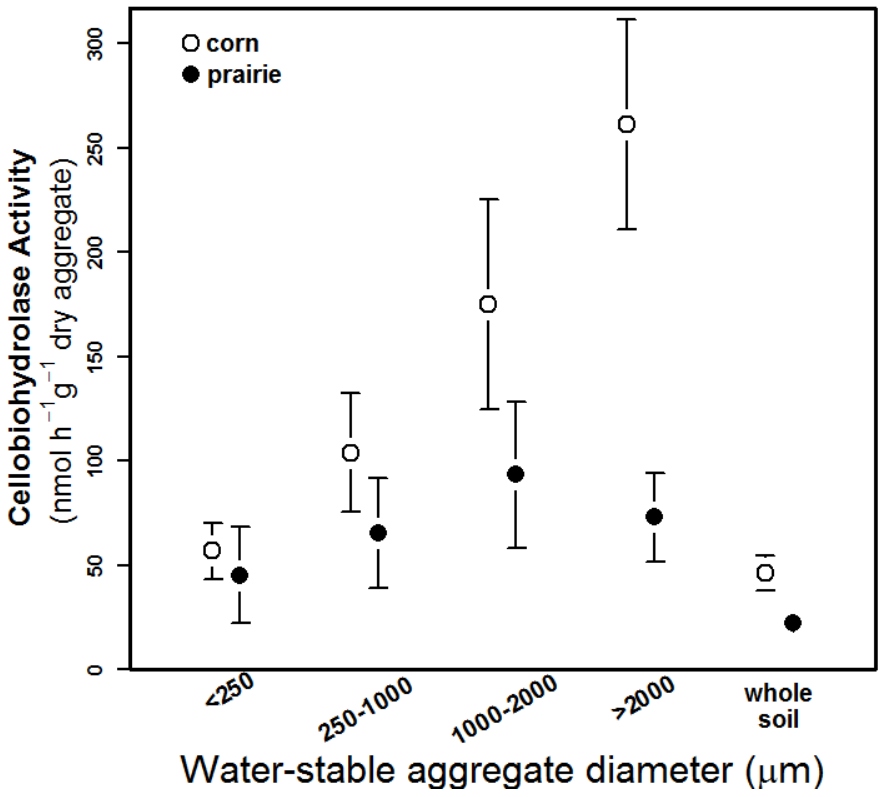

Figure 2.3: Mean aggregate potential enzyme activity, nmol h^{-1} g^{-1} dry aggregate (± 1 SE) of cellobiohydrolase in wet-sieved aggregates isolated from corn (open circles) and prairie (filled circles) ecosystems. Symbols are off-set to improve visual clarity. Error bars on whole soil points are smaller than the symbol. Large macroaggregates (>2000 μm) exhibited greater activity than microaggregates (<250 μm) and whole soil ($P<0.02$) and soil from the corn system had greater cellobiohydrolase activity than prairie ($P<0.005$).

Tables

Table 2.1: Mean aggregate potential enzyme activity (± 1 SE) measured within aggregate fractions isolated by three methods: wet-sieving, dry-sieving, and optimal moisture-sieving. Values are averaged across ecosystem type. Enzymes measured were the N-cycling enzyme N-acetyl-glucosaminidase (NAG) and C-cycling enzymes β-glucosidase (BG), β-xylosidase (BX), and cellobiohydrolase (CB). Different letters denote statistically different means among fractions within a sieving method (α=0.05).

Aggregate Method	**Aggregate Fraction**	**Potential Extracellular Enzyme Activity (nmol h^{-1} g^{-1} dry aggregate)**			
		NAG	**BG**	**CB**	**BX**
Wet-sieving	>2000 µm	$118^{A} \pm 29$	$443^{A} \pm 106$	$167^{A} \pm 45$	$76^{A} \pm 23$
	1000-2000 µm	$117^{AB} \pm 37$	$366^{A} \pm 76$	$134^{A} \pm 31$	$102^{A} \pm 52$
	250-1000 µm	$43^{B} \pm 6$	$234^{A} \pm 37$	$84^{A} \pm 18$	$23^{B} \pm 4$
	<250 µm	$34^{B} \pm 6$	$208^{A} \pm 50$	$51^{AB} \pm 12$	$23^{B} \pm 6$
	Whole soil	$31^{B} \pm 5$	$153^{B} \pm 30$	$34^{B} \pm 6$	$21^{B} \pm 5$
Dry-sieving	>2000 µm	35 ± 6	169 ± 22	43 ± 5	17 ± 2
	1000-2000 µm	21 ± 6	156 ± 23	45 ± 7	20 ± 3
	250-1000 µm	28 ± 6	153 ± 25	39 ± 6	17 ± 1
	<250 µm	26 ± 7	129 ± 25	35 ± 6	19 ± 4
	Whole soil	31 ± 5	153 ± 30	34 ± 6	21 ± 5
Optimal moisture sieving	>2000 µm	16 ± 3	98 ± 12	29 ± 4	17 ± 6
	1000-2000 µm	23 ± 3	132 ± 21	41 ± 4	18 ± 2
	250-1000 µm	26 ± 4	147 ± 31	47 ± 9	25 ± 7
	<250 µm	17 ± 5	131 ± 37	46 ± 15	20 ± 7
	Whole soil	24 ± 4	165 ± 31	39 ± 6	17 ± 3

Table 2.2: Mean proportional potential enzyme activity (± 1 SE) measured within aggregate fractions isolated by three methods: wet-sieving, dry-sieving, and optimal moisture-sieving. Values are averaged across ecosystem type. Enzymes measured were the N-cycling enzyme N-acetyl-glucosaminidase (NAG) and C-cycling enzymes β-glucosidase (BG), β-xylosidase (BX), and cellobiohydrolase (CB). Different letters denote statistically different means among fractions within a sieving method (α=0.05).

Aggregate Method	**Aggregate Fraction**	**Potential Extracellular Enzyme Activity (nmol h^{-1} g^{-1} dry soil)**			
		NAG	**BG**	**CB**	**BX**
Wet-sieving	>2000 µm	8±3	24±5^{B}	9±2	7±3
	1000-2000 µm	10±4	32±7^{B}	11±3	9±4
	250-1000 µm	8±1	45±7^{B}	15±3	4.6±0.8
	<250 µm	15±2	91±22^{A}	22±5	10±3
	Whole soil	31±5	153±30	34±6	21±5
Dry-sieving	>2000 µm	22±4^{A}	106±13^{A}	27±3^{A}	11±1^{A}
	1000-2000 µm	3±1^{B}	24±5^{B}	6±1^{B}	3.3±0.8^{B}
	250-1000 µm	4±2^{B}	22±3^{B}	5.5±0.8^{B}	2.8±0.6^{B}
	<250 µm	1.5±0.5^{C}	6±2^{C}	1.7±0.4^{C}	1.0±0.3^{C}
	Whole soil	31±5	153±30	34±6	21±5
Optimal moisture sieving	>2000 µm	9±2^{A}	61±7^{A}	18±3^{A}	11±4^{A}
	1000-2000 µm	3.9±0.5^{B}	22±4^{B}	7.1±0.8^{B}	3.0±0.4^{B}
	250-1000 µm	5±1^{B}	26±5^{B}	8±1^{B}	4.2±0.8^{B}
	<250 µm	0.06±0.03^{C}	0.5±0.2^{C}	0.15±0.06^{C}	0.05±0.02^{C}
	Whole soil	24±5	165±31	39±6	17±3

Table 2.3: Mean percent recovery (± 1 SE) of extracellular enzyme activity within soil aggregates isolated by three methods: wet-sieving, dry-sieving, and optimal moisture-sieving. Percent recovery was calculated as weighted proportional sum of activity divided by whole soil activity. Different letters denote statistically different mean recovery between methods (α=0.05).

Aggregate Method	Cropping System	% Recovery of Total Potential Extracellular Enzyme Activity			
		NAG	BG	CB	BX
Wet-sieving	Corn	122±18%	102±17%	155±18%	126±50%
	Prairie	147±44%	92±19%	131±15%	198±81%
	Mean recovery	134±23%	102±10%	148^{A}±11%	150±44%
Dry-sieving	Corn	75±10%	78±21%	100±21%	70±26%
	Prairie	100±33%	130±57%	125±37%	128±51%
	Mean recovery	91±16%	114±30%	120^{B}±18%	108±28%
Optimal moisture sieving	Corn	117±64%	66±24%	82±19%	102±29%
	Prairie	102±24%	71±15%	108±4%	124±33%
	Mean recovery	108±32%	72±12%	92^{B}±9%	109±20%

Supplemental Information

Table 2.4S: Mean aggregate potential enzyme activity (± 1 SE) measured within wet-sieved soil aggregates in 2-year old prairie and corn ecosystems.

Aggregate Source	Aggregate Fraction	Potential Extracellular Enzyme Activity (nmol g^{-1} dry aggregate h^{-1})			
		NAG	BG	CB	BX
Wet-sieving: Corn	>2000 µm	154±58	653±137	261±50	82±14
	1000-2000 µm	83±38	347±134	174±50	155±110
	250-1000 µm	42±5	254±50	103±29	22±4
	<250 µm	39±8	223±29	57±14	22±2
	Whole Soil	37±9	213±37	46±8	29±10
Wet-sieving: Prairie	>2000 µm	83±18	233±56	73±21	70±52
	1000-2000 µm	151±70	386±117	93±35	50±19
	250-1000 µm	45±14	214±67	65±26	24±8
	<250 µm	30±10	194±112	45±23	24±15
	Whole Soil	25±6	94±23	22±4	13±3

Table 2.5S: Measures of variance of observed aggregate potential extracellular enzyme activity within aggregate isolated by three methods: wet-sieving, dry-sieving, and optimal moisture-sieving. Different letters denote statistically different mean variance measures between sieving methods (α=0.05). CAPITAL letters are comparison among coefficient of variation (CV); lowercase letters are comparison among standard error (SE). Data were averaged across ecosystem type.

Aggregate Method	Variance Statistic	Variance in Potential Extracellular Enzyme Activity			
		NAG	BG	CB	BX
Wet-sieving	CV	55	55	60^{A}	81
	SE	17	60	22^{a}	81
Dry-sieving	CV	60	44	43^{B}	44
	SE	6	25	6^{b}	44
Optimal moisture sieving	CV	50	49	43^{B}	64
	SE	4	26	7^{b}	64

Table 2.6S: Mean (± 1 SE) aggregate potential enzyme activity in frozen and fresh wet-sieved aggregate fractions averaged across ecosystem type. There was no statistical difference in measured enzyme activity in fresh and frozen wet-sieved aggregate (P>0.3).

Aggregate Method	Aggregate Fraction	Potential Extracellular Enzyme Activity (nmol g^{-1} dry aggregate h^{-1})			
		NAG	BG	CB	BX
Wet-sieving: Fresh	>2000 µm	21±4	118±15	38±10	12±3
	250-2000 µm	10±1	72±14	22±3	9±2
	53-250 µm	4.5±0.7	58±14	20±5	8±3
	<53 µm	5±1	48±7	10±3	4.8±0.9
Wet-sieving: Frozen	>2000 µm	16±2	126±18	38±5	10±1.5
	250-2000 µm	11±2	79±9	24±4	7.2±0.6
	53-250 µm	6.8±0.8	55±11	14±4	6±1
	<53 µm	6±1	43±6	6±1	5.0±0.7

CHAPTER III

SOIL STRUCTURE AND SEASONALITY AFFECT MICROBIAL ACTIVITY IN MANAGED GRASSLANDS

A paper submitted to *Ecosphere*

Elizabeth M. Bach and Kirsten S. Hofmockel

Abstract

Ecological interactions between soil microorganisms and organic matter vary spatially and temporally, influencing cycling and storage of carbon (C) and nitrogen (N) critical to land-atmosphere biogeochemistry. Some studies have characterized extracellular enzyme activity within soil structures (aggregates); however, seasonal variation in decomposition at the micro-scale remains virtually unknown, particularly in contrasting ecosystems. We address variation in soil microbial extracellular enzyme activity spatially across soil aggregates and temporally across two growing seasons in three anthropogenic grassland ecosystems: *Zea mays* L. (corn) agroecosystem, fertilized and unfertilized reconstructed tallgrass prairie. We measured potential N-acetyl-glucosaminidase (NAG), β-glucosidase (BG), β-xylosidase (BX), and cellobiohydrolase (CB) enzyme activity. Independent of ecosystem or sampling date, NAG activity was greatest in large macroaggregates (>2000 μm) and CB activity was greatest in microaggregates (<250 μm). In corn monocultures, soil aggregates turned over early in the growing season, followed by increasing, albeit low, enzyme activity throughout the growing season. Aggregate turnover in prairie systems was driven by precipitation events and seasonal spikes in enzyme activity corresponded with aggregate turnover events.

These results indicate soil aggregation can play an important role mediating microbe-substrate interactions in ecosystems and these interactions can affect quantified differences in C and N cycling between ecosystems.

Keywords: tallgrass prairie, extracellular enzyme activity, aggregates, optimal moisture, agroecosytems, soil structure

Introduction

Microorganisms drive the transfer of energy and nutrients from decaying organic material into living components of ecosystems via the production of extracellular enzymes (Falkowski *et al.*, 2008). In terrestrial systems, the heterogeneous distribution of microorganisms and organic matter in soils regulate decomposition and nutrient mineralization (Ettema & Wardle, 2002). Therefore, spatial and temporal variation in microbial habitat conditions profoundly influence microbial activities that drive cycling of carbon (C) and nitrogen (N) at the ecosystem scale. Homogenization of soil samples structurally (Young & Crawford, 2004), and omission of seasonal microbial responses (Schmidt *et al.*, 2007), may limit measurements and predictions of microbial-scale habitat factors affecting C and N storage within and between ecosystems.

Spatially, microbial interactions are strongly influenced by soil structure, specifically bulk density and the hierarchical distribution of soil aggregate fractions including macroaggregates (250-2000 μm) and microaggregates (53-250 μm) (Tisdall & Oades, 1982). In turn, soil aggregates influence soil organic matter (SOM) storage and decomposition in many ways. Organic matter becomes physically enmeshed within larger aggregates, and therefore protected from decomposition by soil organisms outside the

aggregate (Six *et al.*, 2000b). Within macroaggregates, SOM and soil particles fuse, forming microaggregates. Macroaggregates naturally turn-over in soils (Oades, 1993) while microaggregates are stable for decades to centuries (Jastrow, 1996). As such, soil aggregate turnover is one mechanism driving fluctuations in soil physical habitat. Seasonal patterns of physical changes in the soil environment, including aggregates, directly affect soil microbial processing of organic matter and ecosystem-level cycling of C and N (Schimel & Schaeffer, 2012).

Temporally, soil microbial biomass and activity change across seasons (Lipson *et al.*, 1999) and this response is likely to vary between ecosystems. Observations from alpine and forest ecosystems support a generalized model of temporal partitioning of N assimilation and mineralization between microbes and plants (Schmidt *et al.*, 2007). Mechanisms contributing to seasonal variation in microbial nutrient and C cycling include microbial community succession (Monson *et al.*, 2006), physiological adjustments (Schimel *et al.*, 2007), and availability of plant litter and root exudates (Kaiser *et al.*, 2011). However, previous work has not explicitly considered the role of soil physical habitat on these temporal patterns, or the effect of direct N inputs as an ecosystem management practice. In contrast to previous work investigating N addition in the context of increasing atmospheric deposition (Schmidt *et al.*, 2004), agroecosystems receive large inputs of inorganic N to maximize plant production and lose large quantities of nutrients to surrounding aquatic ecosystems. Explicit understanding of spatial and temporal controls on microbial cycling of C and N in agroecosystems and managed grasslands could advance our understanding of plant-microbe partitioning of nutrient

cycling in general, and provide insight to balance agronomic and ecological functioning in managed ecosystems.

In this study, we examined spatial and temporal drivers of soil extracellular enzyme activity in three ecosystems managed for aboveground biomass production at a single experimental site: *Zea mays* L. (corn) row-crop agroecosystem, restored diverse tallgrass prairie, and fertilized restored tallgrass prairie. These three systems represent a gradient of high N, low C inputs in corn systems and low N, high C inputs in unfertilized prairie systems. Row-crop agroecosystems are also less aggregated than restored prairies (Bach *et al.*, 2010), allowing comparison of seasonal differences in soil physical habitat among the ecosystems. We hypothesized unfertilized prairie systems would support highest extracellular enzyme activity, which would peak late in the growing season due to greater root inputs and plant senescence (Dietzel *in prep*). Nitrogen addition in fertilized prairies would suppress extracellular enzyme activity overall and shift seasonal peaks to earlier in the season due to greater cool-season (C_3) plant cover (Jarchow & Liebman, 2013). Corn systems were expected to have the lowest extracellular enzyme activity, peaking at plant senescence due to minimal root biomass and high concentrations of inorganic N. Among soil aggregates, we predicted extracellular enzyme activity would be greatest in large macroaggregates (>2000 μm) and least in microaggregates (<250 μm) due to greater labile organic matter content in large macroaggregates.

Materials and Methods

Study site

Soil was collected from the Iowa State University Comparison of Biofuel Systems (COBS) experimental site in Boone County, IA (41°55'14.42"N, 93°44'58.96"W, for more details, refer to Jarchow and Liebman (2013)). Soils consisted of loams in the Nicollet (Fine-loamy, mixed, superactive, mesic Aquic Hapludoll) and Webster (Fine-loamy, mixed, superactive, mesic Typic Endoaquoll) series with less than 3% slope. Sand content ranged from 27% to 53% across the site and clay content ranged from 17% to 32%. We sampled three experimental agroecosystem treatments: no-till continuous corn (*Zea mays* L.; fertilization rates determined by spring soil nutrient analysis), planted tallgrass prairie, and fertilized planted tallgrass prairie (84 Kg N ha^{-1} $year^{-1}$). Both planted prairie systems were planted in 2008 with the same seeding mixture of 31 native species and were harvested annually in October for bioenergy feedstock production. There were four blocks per treatment, each containing one 27 m x 61 m plot of each planting treatment in a randomized complete block design (n=4). Soil physical properties at each sampling date are reported in Table 3.1.

Soil Sampling & Aggregate Fractionation

Soils were sampled from the top 10 cm of soil using a 5.5 cm diameter slide-hammer soil coring device (Giddings Machine Company, Windsor, CO). Three intact soil cores were collected from each plot at each sampling time. The mass of each core was used to calculate bulk density before being gently broken up along natural points of weakness and passed through an 8 mm sieve. Replicated cores were combined into one composite sample for each plot. A 10 g sub-sample of soil was removed immediately and

dried at 105°C for 24 hours to determine field fresh gravimetric water content. The remaining soil was prepared for soil aggregate isolation using an optimal moisture approach to standardize soil moisture content and minimize disturbance to microbial communities (Bach & Hofmockel, 2014). Cold-dried soil was placed on a stack of sieves and shaken on a circular sieve shaker (CSI Scientific) at 200-250 rpms for three minutes. Soil was gently removed from each sieve and weighed to determine the mass distribution of aggregates into the following fractions: large macroaggregates (>2000 μm; LM), medium macroaggregates (1000-2000 μm; MM), small macroaggregates (250-1000 μm; SM), and microaggregates (<250 μm; micro). Mean weighted diameter (MWD) of soil aggregates was calculated according to van Bavel (1949). Changes in proportional distribution of soil mass among aggregate fractions between sampling months were interpreted as turnover/reformation of aggregates. Sub-samples of each aggregate fraction were used to determine gravimetric water content, extracellular enzyme activity, and total C and N.

Aggregate Enzyme, C, and N Analysis

Extracellular soil enzyme assays were modified from DeForest (2009) using 4-Methylumbelliferyl (MUB)-linked substrates for N-acetyl-glucosaminidase (NAG) and β-glucosidase (BG), β-xylosidase (BX), and cellobiohydrolase (CB). Briefly, 1 g of frozen soil aggregates was suspended in 125 mL sodium acetate buffer with pH adjusted to median of soils (range 6.4-7.6). Slurries were pipetted into 96-well black micro-plates, and enzyme activities were determined by adding 4-Methylumbelliferyl (MUB)-linked substrates for N-acetyl-glucosaminidase (NAG) and β-glucosidase (BG), β-xylosidase (BX), and cellobiohydrolase (CB) for a final concentration of 40 mM. Assays

were incubated in the dark for 2 hours, reactions stopped with 10μL 0.5 M NaOH, and solution optical density determined fluorometrically at 450 nm on a microplate reader (BioTek, Winooski, VT). In 2012, slight modifications were made to the enzyme assay protocol to reduce variability between analytical replicates. Enzyme concentrations were increased to 400 μM and soil-enzyme slurries were incubated in 5 mL tubes before being transferred to 96 well plates for fluorometric analysis. Re-analysis of a sub-set of 2011 samples with the modified protocol showed no changes in relationships detected in the original dataset. Original 2011 enzyme activity was greater than rerun samples, indicating enzyme activity had degraded in the additional 18 months of storage at -20° C, so we proceeded with the original 2011 and 2012 datasets despite changes made in the assay protocol, and no direct statistical comparisons were made between the 2011 and 2012 data. For all samples, absolute potential enzyme activity (nmol h^{-1} g^{-1} dry aggregates) was calculated and reported as described by German *et al.* (2012b). Total C and N were determined for each aggregate fraction at each sampling date. A sub-sample of isolated aggregates was dried at 60°C for 48-60 hours, ground, and dry combusted in a Thermo Flash 1112 CN analyzer (Thermo Corp, Lakewood, NJ).

Statistical analyses

Potential enzyme activities and total C and N of aggregate fractions were analyzed using a modified split-plot ANOVA, accounting for the non-independence of aggregate and plot (Briar *et al.*, 2011), with treatments as fixed effects and block as a random effect. For all variables, intra-annual responses differed between 2011 and 2012. Therefore, analysis of complete 2011 and 2012 data sets was performed independently. Sampling date*ecosystem was the only interaction detected in the full factorial model.

Thus, all other interaction terms were removed from the model. Means were compared using Tukey's honestly significant difference (α=0.05). Natural log transformations were performed to satisfy assumptions of normality when needed. Models were run using PROC MIXED in SAS v. 9.2 (SAS Institute, Cary, NC).

Results

Intra-annual turnover of soil aggregate and enzyme activity differed between 2011 and 2012 due to drought conditions in 2012 (Appendix A). Dry periods increase aggregate stability and precipitation events can disrupt macroaggregates, increasing bulk density as soil particles fill macropores. Accordingly, we observed more subtle shifts in aggregate turnover across the 2012 growing season compared to 2011 (Fig. 3.1, Table 3.1). In 2011, both prairie systems were most aggregated in June and the corn system was most aggregated in May and June (mean weighted diameter (MWD); $P \leq 0.002$, Fig. 3.1, Table 3.1). Bulk density in all ecosystems was greatest in June ($P=0.0001$; Table 3.1), and differences between ecosystems were much smaller ($P=0.0003$). In 2012, seasonal changes in aggregation were much smaller than in 2011. Fertilized prairie soil was most aggregated in August and least aggregated in May and July, converse to corn soil aggregation ($P=0.0001$, Fig. 3.1, Table 3.1). Aggregate turnover, which had little effect on bulk density in 2012, was consistent in corn systems across the growing season, but was greatest in both prairie systems in October ($P=0.02$, Table 1). In each month except May, fertilized prairie contained more LMs and less SMs than corn systems ($P \leq 0.006$). Overall fertilized prairie had greatest MWD, 3.5 mm, and corn the least, 3.0 mm ($P<0.0001$). In both years and all cropping systems, the mass of SM (250-1000 μm)

fractions increased when the proportion of LM mass decreased (Fig. 3.1), indicating disruption of LMs releases SMs during aggregate turnover.

Aggregate turnover leading to low MWD, along with temperature and soil moisture, corresponded with seasonal peaks in extracellular enzyme activity. In 2011, corn systems reached maximum enzyme activity in August for all enzymes ($P=0.0001$). In contrast, unfertilized prairies reached peak enzyme activity in July. In fertilized prairies, NAG sustained maximum activity in July and August (Fig. 3.2), but C-degrading enzymes BG, CB, and BX reached peak activity in July (Appendix B). Averaged across all sampling dates in 2011, C-cycling enzyme activity was greater in fertilized prairie than the other ecosystems. In 2012, enzyme activity in corn systems exhibited minimal changes across the growing season; NAG activities were consistent across the entire growing season (Fig. 3.2) and C-cycling enzyme activity (BG, CB, BX) was greatest in October (Appendix B). Enzyme activity in unfertilized prairies was least in May, and similar at all other sampling dates. Fertilized prairie systems exhibited very high enzyme activity in August and October, much higher than other systems at those dates. Peak enzyme activity and high MWD in October was likely driven by an interaction of increased soil moisture that month with post-senescence plant inputs. Ecosystem differences were likely influenced by plant inputs, which were relatively low in corn systems, and microbe available N, which may have limited enzyme production in unfertilized prairie systems with internal cycling of plant N. Among aggregates, enzyme activity varied consistently between fractions in 2012. In LMs, NAG activity was 42% greater than in micros ($P<0.02$), but CB activity was 22% greater in micros than LMs ($P=0.03$).

Across all sampling dates, micros had less total C content than SMs, and SMs had less total C than medium and LMs ($P<0.0001$, Fig. 3.3). Total N did not vary between aggregate fractions, but aggregate C:N ratios decreased from 13.2 to 12.2 as aggregate size fraction decreased from LMs (>2000 μm) to micros ($P<0.0001$, Fig. 3.3). When potential enzyme activities were expressed per g C in each aggregate fraction, C specific enzyme activity did not differ between aggregate fractions (Table 3.3S).

Discussion

Seasonal dynamics of soil aggregation and microbial extracellular enzyme activity varied between the managed grassland ecosystems investigated in this study, but differences in enzyme activity among soil aggregate fractions were consistent and driven by aggregate C content. Turnover of aggregates during the growing season corresponded with increases of extracellular enzyme activity, indicating seasonal changes in the soil physical habitat contribute toward ecosystem differences in microbial activity. Together, these results suggest that seasonal shifts in weather, plant inputs, and soil aggregation can affect extracellular enzyme activity, potentially influencing the magnitude and direction of ecosystem differences in microbial C and N cycling.

Temporally, soil aggregation exhibited seasonal dynamics that support previous estimates of one month macroaggregate turnover time (Plante & McGill, 2002), but varied between ecosystems, especially in 2011. In both years, corn systems experienced aggregate disruption between May and July sampling dates, with slight aggregate rebuilding in August. Aggregate disruption early in the growing season in corn systems, but not prairie was likely driven by an interaction of management activities and

precipitation. Aggregate disruption in May 2012 despite the lack of precipitation indicates reduced root inputs, and potentially wheel traffic associated with no-till corn agroecosystem management leaves soil more susceptible to aggregate disruption. By contrast, increased aggregation and reduced magnitude of turnover events in both prairie systems supports previous work emphasizing the role of root networks and microbial activity in stabilizing soil aggregates (Tisdall & Oades, 1982; Six *et al.*, 2004). Aggregate turnover contributed to ecosystem-specific seasonal changes in soil bulk density, underscoring that bulk density is not a static soil measure (Lee *et al.*, 2009). Aggregate turnover events also corresponded with peak enzyme activity. In 2011, peak enzyme activity in July in both prairie systems corresponded with a major disruption of LMs. Release of labile organic matter formally protected in LMs may have stimulate enzyme activity in July and lead to the formation of new LMs later in the growing season (Six *et al.*, 2006).

Spatial variation in extracellular enzyme activity between soil aggregate fractions in all ecosystems and across all sampling dates was driven by aggregate C content. Greater activity of NAG in LMs and elevated C-cycling enzyme activity (BG, CB) in SMs and micros is consistent with previous work (Bailey *et al.*, 2012a). Increased NAG enzyme activity may be attributable to increased demand for C in LMs because NAG cleaves both a glucose and amine group from more complex chains (Fansler *et al.*, 2005). Furthermore, the C:N ratio of soil aggregates increased with increasing aggregate diameter, so there may be greater microbial demand for N in LMs. Likewise, lower C:N ratios in micros may be driving increased CB activity, as observed by Nie *et al.* (2014). Equal C-specific enzyme activity across aggregate fractions indicates total C substrate

drives enzyme activity in aggregates. Although aggregate C content and C specific enzyme activities were consistent, turnover of soil aggregates changed the spatial distribution of aggregates and associated enzyme activities across growing seasons, suggesting a physical mechanism for dynamic seasonal ecosystem-level cycling of C (and N).

Soil moisture and plant growth phase also contributed to observed seasonal fluctuations in enzyme activity. Because a spike in enzyme activity was observed in all systems in October 2012, partial release from drought conditions is the likely driver of this response, consistent with previously observed enzyme responses to soil moisture fluctuation (Zeglin *et al.*, 2013). Peaks in C-cycling enzyme activity were also observed in fertilized prairie systems in July 2011 and 2012. At this experimental site, fertilized prairie systems have greater cover of cool-season (C_3) plants than unfertilized prairie systems (Jarchow & Liebman, 2013); thus, July enzyme activity may be responding to fine root turnover and senescence of cool season plants. Sustained high levels of enzyme activity in unfertilized prairie systems into August and September could be the result of maximum warm-season (C_4) root growth and exudation, consistent with previous studies of microbial biomass in temperate grasslands (Garcia & Rice, 1994). Extracellular enzyme activity in corn monocultures (annual C_4 grass *Zea mays*) increased with plant growth, and was likely limited by low root biomass and allocation of plant C to grain production.

Although differences in enzyme activity and soil aggregation between ecosystems were not consistently observed at each sampling time, when averaged across both 2011 and 2012 growing seasons, fertilized prairie systems had greatest activity and

aggregation, contradicting previous work in unmanaged ecosystems (Schmidt *et al.*, 2004; Sinsabaugh *et al.*, 2005). Interestingly, unfertilized prairie systems had the greatest standing root biomass (Dietzel, unpublished data), suggesting that N availability is stimulating enzyme production beyond root-derived substrates (Yao *et al.*, 2011). Conversely, low availability of root C substrates likely limited enzyme activity in N-rich corn systems (Hargreaves & Hofmockel, 2014). These differences were not evident at every sampling date, but by evaluating the entire growing season we were able to more accurately quantify differences in C and N cycling at the ecosystem scale.

The key conclusions drawn from these data are 1) soil structure (aggregates) affects microbial decomposition of organic matter through extracellular enzyme activity, and 2) ecosystem type and management affect seasonal turnover of aggregate fractions and fluctuations in extracellular enzyme activity, which results in greater microbial stabilization and physical protection of soil organic matter in prairie ecosystems, especially fertilized prairies. Soil aggregation can play an important role mediating microbe-substrate interactions in ecosystems and further consideration of ecosystem processes like extracellular enzyme activity at the aggregate-scale could expand our limited understanding of soil ecosystem processes in a wide range of habitats. In turn, ecosystem and weather strongly influenced soil aggregate turnover. This underscores the importance of *when* soil is sampled as ecosystem differences in enzyme activity and aggregation were not consistent across the growing season. These data indicate seasonal dynamics in extracellular enzymes can influence ecosystem-level C and N cycling and that physical soil structure contributes to these seasonal dynamics within managed grasslands.

Acknowledgments

Funding was provided by the Agriculture and Food Research Initiative of the USDA National institute of Food and Agriculture (NIFA), grant number # 20116700330364 to KSH and an Iowa State University Plant Science Institute Fellowship to EMB. Giselle Narvaez, Becca Luzbetak, Kira Murray, Erin Frankson, Austin Putz, Eric Asbe, Megan Barthalomew, Dade McBrayer, and Mark Anthony provided assistance in the field and laboratory. Richard Cruse, Sarah Hargreaves, Alison King, Ryan Williams, and Brian Wilsey provided valuable feedback on drafts of the manuscript. Authors declare no conflicts of interest.

Literature Cited

Bach, E. M., S. G. Baer, C. K. Meyer, and J. Six. 2010. Soil texture affects soil microbial and structural recovery during grassland restoration. Soil Biology & Biochemistry 42:2182-2191.

Bach, E. M. and K. S. Hofmockel. 2014. Soil aggregate isolation method affects measures of intra-aggregate extracellular enzyme activity. Soil Biology & Biochemistry 69:54-62.

Bailey, V. L., C. L. Bilskis, S. J. Fansler, L. A. McCue, J. L. Smith, and A. Konopka. 2012. Measurements of microbial community activities in individual soil macroaggregates. Soil Biology & Biochemistry 48:192-195.

Briar, S. S., S. J. Fonte, I. Park, J. Six, K. Scow, and H. Ferris. 2011. The distribution of nematodes and soil microbial communities across soil aggregate fractions and farm management systems. Soil Biology & Biochemistry 43:905-914.

DeForest, J. L. 2009. The influence of time, storage temperature, and substrate age on potential soil enzyme activity in acidic forest soils using MUB-linked substrates and L-DOPA. Soil Biology & Biochemistry 41:1180-1186.

Ettema, C. H. and D. A. Wardle. 2002. Spatial soil ecology. Trends in Ecology & Evolution 17:177-183.

Falkowski, P. G., T. Fenchel, and E. F. Delong. 2008. The microbial engines that drive Earth's biogeochemical cycles. Science 320:1034-1039.

Fansler, S. J., J. L. Smith, H. Bolton, and V. L. Bailey. 2005. Distribution of two C cycle enzymes in soil aggregates of a prairie chronosequence. Biology and Fertility of Soils 42:17-23.

Garcia, F. O. and C. W. Rice. 1994. Microbial biomass dynamics in tallgrass prairie. Soil Science Society of America Journal 58:816-823.

German, D. P., M. N. Weintraub, A. S. Grandy, C. L. Lauber, Z. L. Rinkes, and S. D. Allison. 2012. Corrigendum to "Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies" [Soil Biol. Biochem. 43 (2011) 1387–1397]. Soil Biology and Biochemistry 44:151.

Hargreaves, S. K. and K. S. Hofmockel. 2014. Physiological shifts in the microbial community drive changes in enzyme activity in a perennial agroecosystem. Biogeochemistry 117:67-79.

Jarchow, M. E. and M. Liebman. 2013. Nitrogen fertilization increases diversity and productivity of prairie communities used for bioenergy. GCB Bioenergy 5:281-289.

Jastrow, J. D. 1996. Soil aggregate formation and the accrual of particulate and mineral-associated organic matter. Soil Biology & Biochemistry 28:665-676.

Kaiser, C., L. Fuchslueger, M. Koranda, M. Gorfer, C. F. Stange, B. Kitzler, F. Rasche, J. Strauss, A. Sessitsch, S. Zechmeister-Boltenstern, and A. Richter. 2011. Plants control the seasonal dynamics of microbial N cycling in a beech forest soil by belowground C allocation. Ecology 92:1036-1051.

Lee, J., J. W. Hopmans, D. E. Rolston, S. G. Baer, and J. Six. 2009. Determining soil carbon stock changes: Simple bulk density corrections fail. Agriculture Ecosystems & Environment 134:251-256.

Lipson, D. A., S. K. Schmidt, and R. K. Monson. 1999. Links between microbial population dynamics and nitrogen availability in an alpine ecosystem. Ecology 80:1623-1631.

Monson, R. K., D. L. Lipson, S. P. Burns, A. A. Turnipseed, A. C. Delany, M. W. Williams, and S. K. Schmidt. 2006. Winter forest soil respiration controlled by climate and microbial community composition. Nature 439:711-714.

Nie, M., E. Pendall, C. Bell, and M. D. Wallenstein. 2014. Soil aggregate size distribution mediates microbial climate change feedbacks. Soil Biology and Biochemistry 68:357-365.

Oades, J. M. 1993. The role of biology in the formation, stabilization and degradation of soil structure. Geoderma 56:377-400.

Plante, A. F. and W. B. McGill. 2002. Intraseasonal soil macroaggregate dynamics in two contrasting field soils using labeled tracer spheres Soil Science Society of America Journal 66:1285-1295.

Schimel, J., T. C. Balser, and M. Wallenstein. 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology 88:1386-1394.

Schimel, J. and S. M. Schaeffer. 2012. Microbial control over carbon cycling in soil. Frontiers in Microbiology 3.

Schmidt, S. K., E. K. Costello, D. R. Nemergut, C. C. Cleveland, S. C. Reed, M. N. Weintraub, A. F. Meyer, and A. M. Martin. 2007. Biogeochemical consequences of rapid microbial turnover and seasonal succession in soil. Ecology 88:1379-1385.

Schmidt, S. K., D. A. Lipson, R. E. Ley, M. C. Fisk, and A. E. West. 2004. Impacts of chronic nitrogen additions vary seasonally and by microbial functional group in tundra soils. Biogeochemistry 69:1-17.

Sinsabaugh, R. L., M. E. Gallo, C. Lauber, M. P. Waldrop, and D. R. Zak. 2005. Extracellular enzyme activities and soil organic matter dynamics for northern hardwood forests receiving simulated nitrogen deposition. Biogeochemistry 75:201-215.

Six, J., H. Bossuyt, S. Degryze, and K. Denef. 2004. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil & Tillage Research 79:7-31.

Six, J., S. D. Frey, R. K. Thiet, and K. M. Batten. 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Science Society of America Journal 70:555-569.

Six, J., K. Paustian, E. T. Elliott, and C. Combrink. 2000. Soil structure and organic matter: I. Distribution of aggregate-size classes and aggregate-associated carbon. Soil Science Society of America Journal 64:681-689.

Tisdall, J. M. and J. M. Oades. 1982. Organic-matter and water-stable aggregates in soils. Journal of Soil Science 33:141-163.

van Bavel, C. H. M. 1949. Mean weight-diameter of soil aggregates as a statistical index of aggregation. Proceedings Soil Science Society of America 14:20-23.

Yao, H., D. Bowman, and W. Shi. 2011. Seasonal variations of soil microbial biomass and activity in warm- and cool-season turfgrass systems. Soil Biology and Biochemistry 43:1536-1543.

Young, I. M. and J. W. Crawford. 2004. Interactions and self-organization in the soil-microbe complex. Science 304:1634-1637.

Zeglin, L. H., P. J. Bottomley, A. Jumpponen, C. W. Rice, M. Arango, A. Lindsley, A. McGowan, P. Mfombep, and D. D. Myrold. 2013. Altered precipitation regime affects the function and composition of soil microbial communities on multiple time scales. Ecology 94:2334-2345.

Tables

Table 3.1: Soil physical conditions (mean ±1 SE) from corn, fertilized prairie, and prairie ecosystems at each sampling date across two growing seasons (*n*=4).

Ecosystem *Sampling date*	**Gravimetric Water Content**	**pH**	**Soil Temperature (°C)**	**Bulk Density (g soil cm^{-3})**	**Aggregate Mean Weighted Diameter (mm)**
Corn					
2011-May	0.21±0.01	6.5±0.1^{B}	16.8±0.3	1.57±0.03	3.7±0.1^{A}
2011-June	0.12±0.01	6.4±0.1^{B}	18.6±0.3	1.66±0.03	3.7±0.1^{A}
2011-July	0.13±0.01	6.6±0.1^{B}	22.5±0.3	1.39±0.03	2.6±0.1^{B}
2011-Aut	0.18±0.01	7.1±0.1^{A}	21.5±0.3	1.43±0.03	2.9±0.1^{B}
2012-May	0.167±0.009^{A}	5.9^{B}	15.9±0.9	1.21±0.04	3.2±0.1
2012-July	0.122±0.009^{BC}	6.5^{AB}	21.8±0.2	1.16±0.04	2.9±0.1
2012-Aug	0.113±0.009^{C}	6.6^{A}	19.3±0.1	1.12±0.04	3.0±0.1
2012-Sep	0.108±0.009^{C}	6.7^{A}	13.9±0.6	1.24±0.04	2.8±0.1
2012-Oct	0.158±0.009^{AB}	6.6^{A}	.	1.22±0.04	2.9±0.1
Fertilized Prairie					
2011-May	0.19±0.01	7.2±0.1	14.1±0.1	1.51±0.03	3.6±0.1$^{\mathbf{B}}$
2011-June	0.12±0.01	7.2±0.1	17.6±0.2	1.64±0.03	4.3±0.1$^{\mathbf{A}}$

Table 3.1 cont.

2011-July	0.16±0.01	7.1±0.1	20.8±0.1	1.26±0.03	3.2±0.1$^{\mathbf{B}}$
2011-Aug	0.19±0.01	7.3±0.1	20.6±0.2	1.30±0.03	3.3±0.1$^{\mathbf{B}}$
2012-May	0.112±0.009$^{\mathbf{B}}$	7.1	16.0±0.3	0.89±0.04$^{\mathbf{B}}$	3.4±0.1
2012-July	0.151±0.009$^{\mathbf{AB}}$	7.4	21.3±0.3	1.10±0.04$^{\mathbf{A}}$	3.4±0.1
2012-Aug	0.125±0.009$^{\mathbf{B}}$	7.3	18.9±0.2	1.06±0.04$^{\mathbf{AB}}$	3.7±0.1
2012-Sep	0.111±0.009 $^{\mathbf{B}}$	7.2	14.2±0.4	1.05±0.04$^{\mathbf{AB}}$	3.4±0.1
2012-Oct	0.190±0.009$^{\mathbf{A}}$	7.5	.	1.15±0.04$^{\mathbf{A}}$	3.5±0.1
Prairie					
2011-May	0.19±0.01	7.4±0.1	15.1±0.2	1.54±0.03	3.4±0.1^{B}
2011-June	0.12±0.01	7.2±0.1	18.6±0.2	1.64±0.03	4.1±0.1^{A}
2011-July	0.15±0.01	7.0±0.1	23.2±0.3	1.27±0.03	2.8±0.1^{B}
2011-Aug	0.17±0.01	7.3±0.1	21.4±0.3	1.39±0.03	2.8±0.1^{B}
2012-May	0.100±0.009^{C}	7.6	17.3±0.4	0.99±0.04^{B}	3.1±0.1
2012-July	0.146±0.009^{AB}	7.4	22.9±0.4	1.20±0.04^{A}	3.2±0.1
2012-Aug	0.126±0.009^{BC}	7.3	20.1±0.4	1.13±0.04^{AB}	3.2±0.1
2012-Sep	0.110±0.009^{BC}	7.2	14.6±0.5	1.16±0.04^{AB}	3.1±0.1
2012-Oct	0.175±0.009^{A}	7.3	.	1.19±0.04^{A}	3.3±0.1

Table 3.1 Notes: Soil temperature is average of temperature at 5 cm and 10 cm below soil surface. Different letters denote statistical difference between means of sampling date within an ecosystem in each year (α=0.05). Corn systems denoted in plain text, **fertilized prairie denoted in bold**, and *prairie denoted in italics*.

Figures

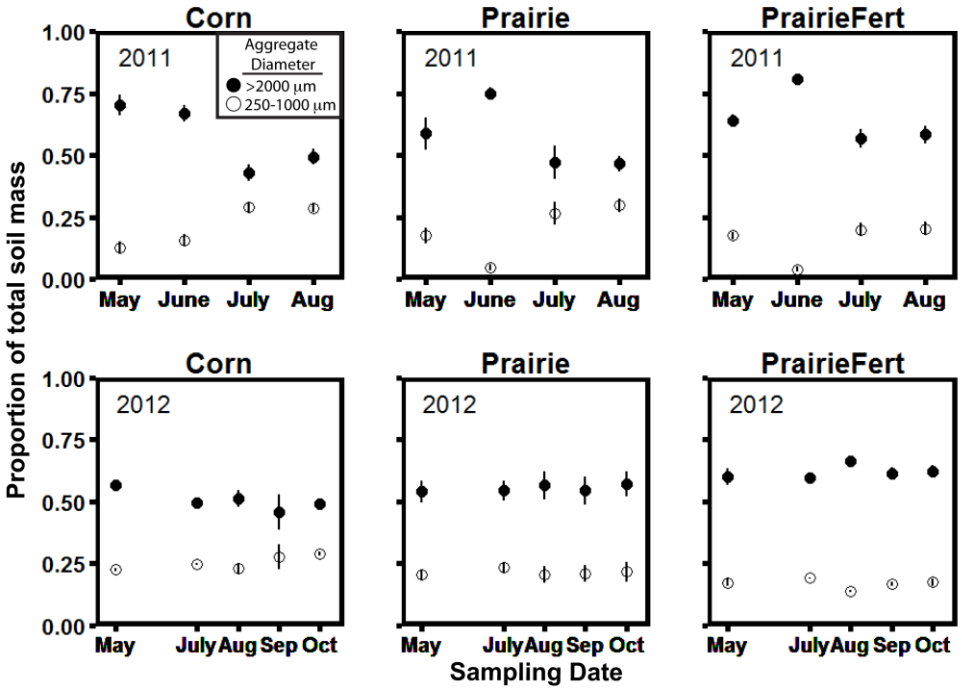

Figure 3.1 Changes in the proportion of total soil mass made up of large macroaggregates (>2000 µm) and small macroaggregates (250-1000 µm) in 2011 and 2012 growing seasons. In some cases, symbols are larger than error bars (±1 SE, *n*=4). Capital letters denote differences among large macroaggregates, lowercase letters represent differences among small macroaggregates within each ecosystem (α=0.05). Mass lost from large macroaggregates across the growing season accrued in the small macroaggregate fraction indicating large macroaggregates fall apart into small macroaggregtes.

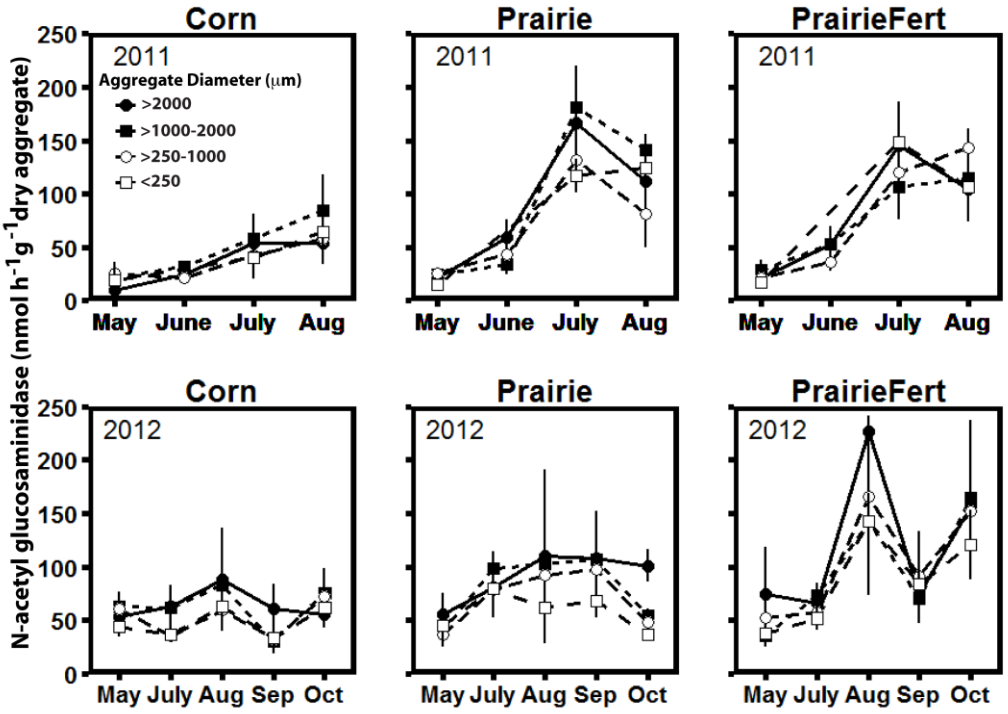

Figure 3.2: Changes in intra-aggregate N-acetyl glucosaminidase activity across two growing seasons in corn, prairie, and fertilized prairie ecosystems. Lines are added for visual clarity only.

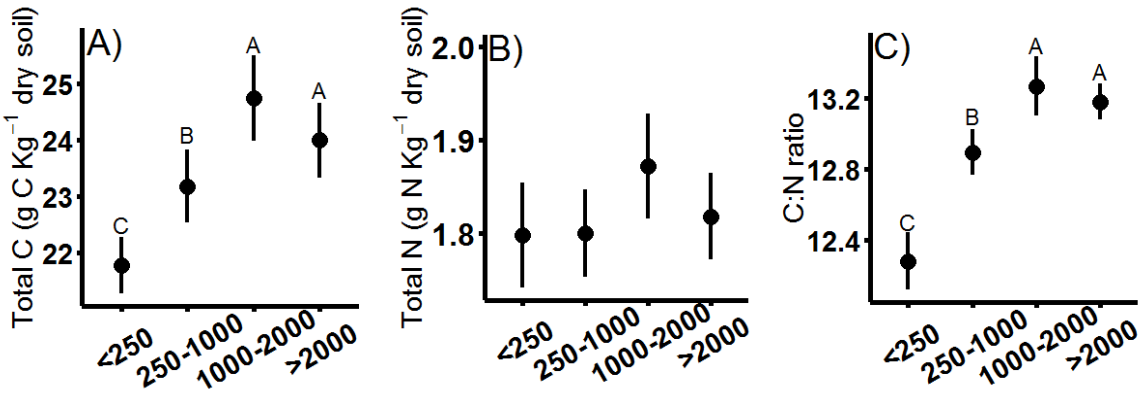

Figure 3.3: Mean (±1 SE) a) total carbon (C), b) total nitrogen (N), and c) C:N of soil aggregates in 2012. Different letters denote statistical difference (α=0.05).

Appendix

Table 3.2: Appendix A Monthly weather summary at Comparison of Biofuel Systems field site for 2011 and 2012 growing seasons and long-term mean.

	Precipitation (mm)			**Air temperature (°C)**		
	2011	**2012**	**1951-2009 mean**	**2011**	**2012**	**1951-2009 mean**
May	145	61	115	15.2	25.9	16.1
June	170	73	124	21.2	27.8	21.2
July	54	60	103	25.6	32.2	23.2
Aug	89	55	106	22.0	28.3	21.9
Sep	50	27	80	15.8	25.3	17.6
Oct	13	60	63	11.9	16.5	11.2
Total	**521**	**337**	**592**			

Figure 3.4: Appendix B Changes in intra-aggregate cellobiohydrolase activity across two growing seasons in corn, prairie, and fertilized prairie ecosystems. Note scale difference between 2011 and 2012. Lines are added for visual clarity only.

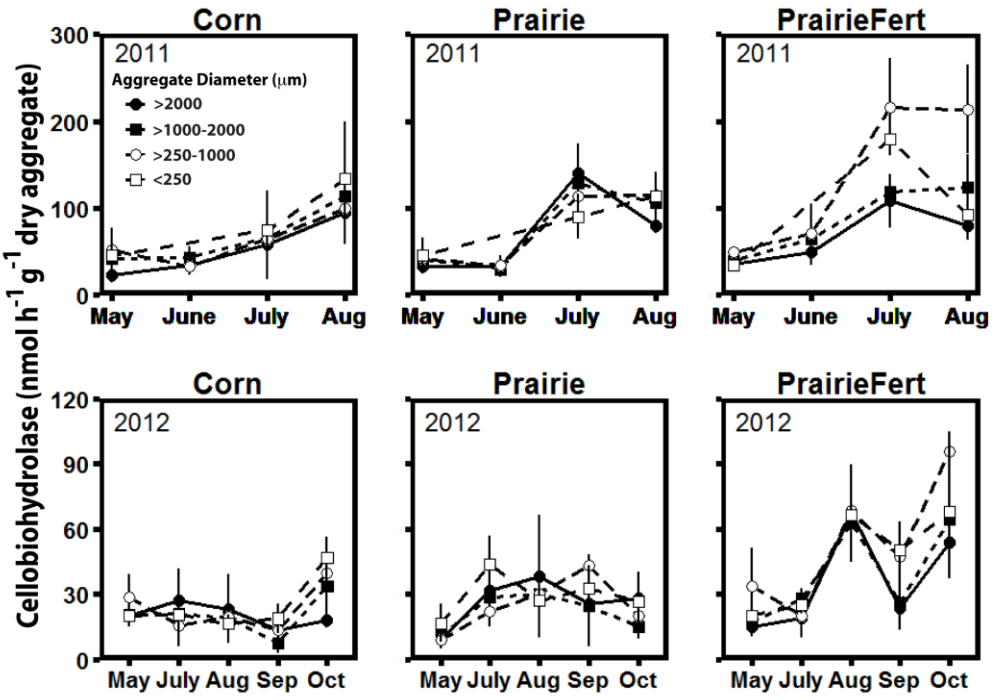

Table 3.3S: Carbon-specific enzyme activity measured within soil aggregates from corn, prairie, and fertilized prairie ecosystems across two growing seasons.

				C-specific enzyme activity (µmol h^{-1} g^{-1} C)							
				BG2011		BX2011		CB2011		NAG2011	
Crop	**Year**	**Date**	**Aggregate Fraction (µm)**	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**
Corn	2011	Aug	>2000	9.90	2.36	2.06	0.23	4.02	0.60	2.31	0.50
Corn	2011	Aug	1000-2000	16.53	2.85	1.88	0.38	4.50	0.79	4.01	1.02
Corn	2011	Aug	250-1000	14.48	2.73	1.58	0.16	4.18	0.71	2.67	0.28
Corn	2011	Aug	<250	15.72	2.54	1.94	0.37	6.43	1.73	3.31	0.53
Corn	2011	July	>2000	14.53	1.71	0.78	0.21	2.27	0.68	2.42	0.55
Corn	2011	July	1000-2000	16.55	2.89	1.24	0.30	2.77	0.93	2.57	0.60
Corn	2011	July	250-1000	12.10	3.81	1.21	0.35	2.83	1.16	1.89	0.43
Corn	2011	July	<250	12.30	3.90	1.05	0.53	3.56	1.24	1.90	0.54
Corn	2011	June	>2000	7.59	1.25	0.68	0.05	1.48	0.11	1.05	0.08
Corn	2011	June	1000-2000	4.39	0.26	0.86	0.20	1.79	0.25	1.35	0.14
Corn	2011	June	250-1000	5.32	0.24	0.85	0.08	1.44	0.17	1.00	0.10

Table 3.3S cont.											
Corn	2011	May	>2000	3.49	0.49	0.47	0.06	1.03	0.14	0.45	0.10
Corn	2011	May	1000-2000	5.01	1.09	0.73	0.06	1.86	0.17	0.95	0.13
Corn	2011	May	250-1000	7.52	1.38	0.93	0.08	2.34	0.30	1.17	0.18
Corn	2011	May	<250	7.65	3.07	0.79	0.32	2.09	0.97	0.86	0.41
Prairie	2011	Aug	>2000	14.77	1.47	1.99	0.02	3.41	0.21	4.93	0.58
Prairie	2011	Aug	1000-2000	11.64	2.34	2.76	0.36	4.58	0.66	6.37	0.75
Prairie	2011	Aug	250-1000	14.87	1.22	2.42	0.40	5.33	0.64	3.67	0.79
Prairie	2011	Aug	<250	22.55	4.66	5.55	0.94	6.40	0.46	7.04	0.62
Prairie	2011	July	>2000	22.26	1.23	3.62	0.27	5.39	0.62	6.64	0.32
Prairie	2011	July	1000-2000	23.04	0.91	5.13	.	5.19	0.57	7.49	0.98
Prairie	2011	July	250-1000	27.94	6.38	2.51	0.97	5.50	1.29	6.29	1.21
Prairie	2011	July	<250	21.89	1.78	3.62	0.35	3.96	0.66	5.22	0.31
Prairie	2011	June	>2000	7.36	1.04	1.05	0.20	1.40	0.27	2.66	0.56
Prairie	2011	June	1000-2000	6.15	0.81	0.80	0.08	1.44	0.23	1.64	0.36
Prairie	2011	June	250-1000	7.73	0.61	1.29	0.12	1.92	0.36	2.29	0.35
Prairie	2011	May	>2000	5.45	0.71	0.91	0.19	1.52	0.13	1.03	0.19

Table 3.3S cont.											
Prairie	2011	May	1000-2000	6.34	0.28	0.81	0.05	1.82	0.21	1.05	0.08
Prairie	2011	May	250-1000	6.26	0.68	1.34	0.26	1.94	0.20	1.35	0.22
Prairie	2011	May	<250	5.51	0.86	1.02	0.27	2.26	0.51	0.78	0.08
PrairieFert	2011	Aug	>2000	14.38	2.02	2.27	0.14	3.06	0.32	4.14	0.73
PrairieFert	2011	Aug	1000-2000	16.22	2.27	2.56	0.38	4.90	1.14	4.60	1.27
PrairieFert	2011	Aug	250-1000	19.70	2.06	3.92	0.28	7.59	0.99	5.63	0.66
PrairieFert	2011	Aug	<250	16.23	1.74	3.46	0.66	4.09	0.35	4.76	0.41
PrairieFert	2011	July	>2000	20.31	1.22	3.01	0.41	4.09	1.12	5.53	1.20
PrairieFert	2011	July	1000-2000	20.26	1.71	3.50	0.77	4.18	0.16	3.76	0.56
PrairieFert	2011	July	250-1000	31.44	9.98	3.94	0.61	9.59	2.56	4.97	0.18
PrairieFert	2011	July	<250	28.02	1.31	6.76	1.99	7.01	0.27	5.81	0.02
PrairieFert	2011	June	>2000	9.35	0.78	1.51	0.25	2.06	0.23	2.20	0.43
PrairieFert	2011	June	1000-2000	11.23	2.12	1.75	0.29	2.49	0.75	2.02	0.41
PrairieFert	2011	June	250-1000	9.43	1.31	1.33	0.16	3.02	0.88	1.59	0.25
PrairieFert	2011	May	>2000	4.77	0.07	0.86	0.11	1.40	0.07	0.90	0.18
PrairieFert	2011	May	1000-2000	5.97	0.17	0.80	0.04	1.51	0.08	1.13	0.21

Table 3.3S cont.											
PrairieFert	2011	May	250-1000	6.08	0.21	1.08	0.17	2.08	0.16	0.95	0.16
PrairieFert	2011	May	<250	4.40	0.38	0.67	0.14	1.33	0.22	0.65	0.07
				BG 2012		**BX 2012**		**CB2012**		**NAG2012**	
Crop	**Year**	**Month**	**Aggregate Fraction (µm)**	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**
Corn	2012	Aug	>2000	22.33	6.15	3.04	1.06	0.94	0.32	3.66	0.94
Corn	2012	Aug	1000-2000	18.82	3.10	2.04	0.40	0.86	0.23	3.74	0.85
Corn	2012	Aug	250-1000	17.78	1.52	1.93	0.26	0.90	0.16	2.78	0.40
Corn	2012	Aug	<250	19.29	3.01	2.13	0.31	0.77	0.11	2.94	0.44
Corn	2012	July	>2000	16.68	5.26	2.24	0.48	1.17	0.35	2.76	0.46
Corn	2012	July	1000-2000	12.46	1.24	2.25	0.26	0.89	0.16	2.75	0.12
Corn	2012	July	250-1000	7.38	1.32	1.46	0.31	0.69	0.20	1.68	0.11
Corn	2012	July	<250	11.73	2.05	1.82	0.33	0.94	0.21	1.73	0.19
Corn	2012	May	>2000	18.23	2.16	2.06	0.19	1.06	0.18	2.71	0.40
Corn	2012	May	1000-2000	19.46	0.86	2.62	0.19	0.95	0.08	2.96	0.28
Corn	2012	May	250-1000	20.75	2.78	2.88	0.54	1.26	0.19	2.70	0.25
Corn	2012	May	<250	16.98	1.39	2.25	0.14	0.97	0.05	2.14	0.26

Table 3.3S cont.											
Corn	2012	Oct	>2000	12.34	0.76	2.21	0.16	0.90	0.07	2.77	0.38
Corn	2012	Oct	1000-2000	20.42	5.57	3.12	0.65	1.34	0.37	3.15	0.41
Corn	2012	Oct	250-1000	20.20	2.40	3.38	0.41	1.76	0.29	3.39	0.45
Corn	2012	Oct	<250	25.21	2.34	4.61	0.52	2.37	0.25	3.15	0.17
Corn	2012	Sep	>2000	22.63	6.57	1.94	0.40	0.65	0.08	3.30	0.91
Corn	2012	Sep	1000-2000	13.72	2.66	1.31	0.26	0.36	0.13	1.56	0.33
Corn	2012	Sep	250-1000	16.23	3.65	1.51	0.26	0.64	0.22	1.60	0.35
Corn	2012	Sep	<250	28.93	8.22	2.65	0.56	0.96	0.23	7.24	3.74
Prairie	2012	Aug	>2000	25.09	7.90	3.20	0.91	1.37	0.51	7.01	1.74
Prairie	2012	Aug	1000-2000	25.48	2.90	3.96	0.48	1.48	0.19	5.24	0.39
Prairie	2012	Aug	250-1000	23.74	3.24	3.66	0.70	1.22	0.26	4.11	0.72
Prairie	2012	Aug	<250	17.11	1.74	2.52	0.23	1.34	0.33	2.91	0.30
Prairie	2012	July	>2000	16.46	2.89	3.44	0.66	1.19	0.18	3.44	0.36
Prairie	2012	July	1000-2000	13.81	1.76	2.31	0.44	1.13	0.31	3.94	0.45
Prairie	2012	July	250-1000	18.44	0.84	2.89	0.22	0.98	0.15	3.54	0.29
Prairie	2012	July	<250	30.28	3.96	5.71	0.80	2.21	0.30	4.05	0.76

Table 3.3S cont.											
Prairie	2012	May	>2000	5.97	1.02	1.03	0.20	0.36	0.07	2.49	0.58
Prairie	2012	May	1000-2000	6.60	1.44	1.12	0.30	0.43	0.14	1.66	0.26
Prairie	2012	May	250-1000	6.64	1.05	1.27	0.24	0.40	0.10	1.78	0.32
Prairie	2012	May	<250	9.63	2.38	1.79	0.46	0.71	0.20	1.99	0.48
Prairie	2012	Oct	>2000	15.23	1.27	2.63	0.31	1.05	0.16	4.26	0.42
Prairie	2012	Oct	1000-2000	10.22	1.05	1.97	0.28	0.55	0.08	2.24	0.20
Prairie	2012	Oct	250-1000	13.81	0.34	2.41	0.35	0.86	0.03	2.19	0.19
Prairie	2012	Oct	<250	17.16	1.87	2.79	0.74	1.30	0.26	2.02	0.19
Prairie	2012	Sep	>2000	15.17	4.12	2.63	0.71	0.90	0.33	4.45	0.77
Prairie	2012	Sep	1000-2000	14.74	2.42	2.83	0.66	0.90	0.19	3.86	0.14
Prairie	2012	Sep	250-1000	22.25	1.84	4.27	0.51	2.02	0.21	4.61	0.43
Prairie	2012	Sep	<250	18.54	1.68	3.43	0.42	1.43	0.21	3.36	0.60
PrairieFert	2012	Aug	>2000	41.48	5.71	5.06	0.76	2.64	0.44	8.80	0.39
PrairieFert	2012	Aug	1000-2000	43.22	8.29	5.98	1.20	3.07	0.51	7.47	1.70
PrairieFert	2012	Aug	250-1000	50.50	7.53	7.06	0.94	3.96	0.88	9.72	2.18
PrairieFert	2012	Aug	<250	39.62	3.93	5.26	0.68	2.58	0.37	5.59	0.52

Table 3.3S cont.											
PrairieFert	2012	July	>2000	13.91	1.75	1.58	0.19	0.69	0.20	2.41	0.24
PrairieFert	2012	July	1000-2000	14.88	1.88	2.40	0.33	0.99	0.10	2.71	0.36
PrairieFert	2012	July	250-1000	16.82	2.47	2.38	0.23	0.80	0.12	2.36	0.42
PrairieFert	2012	July	<250	23.00	2.31	2.66	0.26	1.09	0.08	2.34	0.37
PrairieFert	2012	May	>2000	28.08	11.41	2.87	1.19	1.39	0.55	2.96	1.05
PrairieFert	2012	May	1000-2000	13.23	2.24	1.38	0.19	0.71	0.16	1.47	0.29
PrairieFert	2012	May	250-1000	21.34	5.12	2.57	0.73	1.20	0.28	1.91	0.31
PrairieFert	2012	May	<250	15.37	1.14	1.76	0.17	0.83	0.08	1.60	0.13
PrairieFert	2012	Oct	>2000	26.86	3.00	4.13	0.74	1.93	0.32	5.97	1.18
PrairieFert	2012	Oct	1000-2000	41.38	8.25	4.83	0.82	4.08	1.19	9.73	2.66
PrairieFert	2012	Oct	250-1000	34.48	1.01	6.00	0.71	3.64	0.15	5.86	0.74
PrairieFert	2012	Oct	<250	40.12	5.96	6.25	1.23	3.78	0.67	5.27	0.70
PrairieFert	2012	Sep	>2000	24.30	4.39	2.17	0.43	0.84	0.20	2.79	0.42
PrairieFert	2012	Sep	1000-2000	25.35	5.54	2.45	0.47	1.25	0.36	3.19	0.62
PrairieFert	2012	Sep	250-1000	27.73	7.35	3.24	0.82	1.67	0.43	3.21	1.10
PrairieFert	2012	Sep	<250	34.15	2.38	4.00	0.41	1.95	0.23	3.25	0.50

CHAPTER IV

CARBON AND NITROGEN DRIVE FUNGAL COMMUNITY COMPOSITION ACROSS SOIL AGGREGATES AND LAND MANAGEMENT

A paper to be submitted to *New Phytologist*

Elizabeth M. Bach, Ryan J. Williams, Kirsten S. Hofmockel

Summary

- Soil fungi are strongly influenced by habitat and resources, including plant inputs and soil structure (aggregates).
- This study investigates ecological drivers of fungal community structure across land uses (macro-scale) and across soil aggregates (micro-scale).
- We used the Illumina MiSeq platform to sequence fungal communities from whole soil and aggregate fractions from three bioenergy production systems: reconstructed tallgrass prairie, fertilized reconstructed tallgrass prairie, and continuous maize row-crop cultivation.
- The rarified fungal dataset included 2,046 unique OTUs from 100,000 sequences; Basidiomycota was the most abundant identified phyla.
- Across land uses, maize, unfertilized prairie, and fertilized prairie supported distinct fungal communities, likely driven by plant inputs and fertilizer management.
- Microaggregates (<250 μm) supported more than twice the fungal richness of large macroaggregates (>2000 μm). Whole soil samples contained half the richness of a calculated mass-proportional whole soil weighted sum of aggregate fractions.

Introduction

Plants interact with soil microbial communities directly through mutualisms and parasitism and indirectly for recycling of nutrients from dead organic matter. Fungi are a major component of soil community diversity (Fierer *et al.*, 2007) and the dominant taxa

with regards to biomass (Ekelund *et al.*, 2001; Strickland & Rousk, 2010), contributing on average 70% of total microbial residue carbon (C), which ranges in the tens of mg g^{-1} dry soil (Appuhn & Joergensen, 2006; Joergensen & Wichern, 2008). Scientific understanding of soil fungal taxa and community structure is increasing with application of next-generation sequencing approaches to environmental samples. Recent work has shown soil fungal communities are distinct among plant communities dominated by the Pinaceae family across North America (Talbot *et al.*, 2014), between plant communities in a single habitat (Karst *et al.*, 2013), and across soil depths within a tallgrass prairie (Jumpponen *et al.*, 2010). However, the roles of specific biotic and abiotic factors structuring soil fungal communities at regional and site levels remain largely unexplored.

As the primary energy and nutrient resource for fungi, plant inputs, particularly roots, can profoundly influence fungal community structure (Broeckling *et al.*, 2008; Peay *et al.*, 2013). Because fungi are the primary degraders of lignin, much research attention has been focused on characterizing fungal communities and their functional importance in forest systems e.g. (Talbot *et al.*, 2014; Voříšková *et al.*, 2014). Fungi are believed to play a less important role in grasslands, including agricultural systems, due to reduced lignin inputs (Guggenberger *et al.*, 1994) and increased bacterial abundance in these systems (Hendrix *et al.*, 1986; Six *et al.*, 2006). However, the importance of fungi, especially plant-symbiotic arbuscular mycorrhizal fungi (AMF; phyla Glomeromycota), has long been acknowledged in these systems (Hendrix *et al.*, 1986; Hartnett *et al.*, 1993; Hartnett & Wilson, 1999; Verbruggen *et al.*, 2013). Additionally, managed grasslands and agroecosystems provide excellent study systems in which to investigate how physical and chemical characteristics influence both plant and microbial communities because these factors are commonly manipulated as part of management. As such, investigating fungal community differences between land management on the same soil at the same experimental site provides an opportunity to test how systems level management can interact with edaphic factors to shape fungal communities.

Identifying the dominant factors shaping fungal community structure among ecosystems may require consideration of the fungal habitat from the micro-scale. Much research attention has been given to fungi and their role in soil ecology, at the millimeter scale. Soil fungi exhibit multiple growth forms including macrofungi, filamentous

growth forms, and single-celled yeasts which allow for a diverse community to coexist among niches spanning soil habitats (Drazkiewicz, 1996). Soil aggregates, stable associations of clay, sand, mineral particles, organic matter, roots, and microorganisms, form the basis of soil structure and belowground habitat. Fungi are critical players in aggregate formation and stabilization (Denef *et al.*, 2001; Rillig & Mummey, 2006; Six *et al.*, 2006). Physically, fungal hyphae enmesh silt, clay, and sand particles with bits of organic matter, microaggregates (<250 μm), and other soil particles in a "sticky-string bag" (Oades 1993, Rillig & Mummey 2006). Microaggregates are formed as microbial products and clay particles bind around fine organic matter and as microbes continue to decompose the organic matter within surrounding large macroaggregates (>2000 μm) (Angers *et al.*, 1997; Six *et al.*, 2000a). Large macroaggregates are the largest and least stable aggregate, with an estimated mean turn-over time of weeks (Plante & McGill, 2002). Macroaggregates break apart, releasing stable microaggregates into the soil profile, which can persist in the soil matrix or be incorporated into macroaggregates with plant roots and litter. Microaggregates are highly stable and persist in soils for decades or centuries, protecting mineral-associated C from decomposition (Tisdall & Oades, 1982; Jastrow, 1996; Six *et al.*, 2000a); thus soil aggregation and associated fungi play a critical role in controlling decomposition and nutrient availability for growing plants. Despite this established paradigm, there is limited knowledge as to which fungal taxa are associated with different aggregate fractions and which taxa might be most important in aggregate formation and stabilization.

In this study, we examined soil fungal communities across four soil aggregate fractions under three land management practices at two sampling dates in surface soils (10 cm). The management systems included a continuous maize rotation and two reconstructed tallgrass prairie ecosystems, one receiving inorganic N fertilizer input and one without fertilizer inputs. Our primary goal was to address two key hypotheses testing ecological drivers of fungal community composition at the macro- (m) and micro- (μm) scale:

1) Differentiation of fungal community composition among management systems will occur due to variation in root C inputs and different inorganic N fertilization rates.

2) Roots, which contribute to large macroaggregate formation, will select for distinct fungal communities dominated by a few taxa in large macroaggregates. Fungal presence will be enhanced in large macroaggregates due to their role in aggregate formation and stabilization. In contrast, microaggregates will support several lowly abundant taxa specializing in decomposition of microbially-processed organic matter.

We sequenced fungal internal transcribed tracer (ITS) regions using modified ITS 1F and ITS2 primers (Smith & Peay, 2014) and the Illumina MiSeq platform. Fungal communities were compared between aggregates and between management systems and correlated with soil C and N pools (Bach & Hofmockel, *in review-b*).

Materials & Methods

Study site

Soil was collected from the Iowa State University Comparison of Biofuel Systems (COBS) experimental site located on the South Reynoldson Farm in Boone County, IA (41°55'14.42"N, 93°44'58.96"W); see Jarchow and Liebman (2013) for a detailed site description. The present study includes only three of the experimental agroecosystem treatments: no-till continuous maize (*Zea mays*, corn), planted tallgrass prairie, and fertilized planted tallgrass prairie. Both planted prairie systems were planted in 2008 with the same seeding mixture of 31 native species. Four replicate blocks contain one 27 m x 61 m plot of each planting treatment in a randomized complete block design (total n=12). Soils consisted of loams in the Nicollet (Fine-loamy, mixed, superactive, mesic Aquic Hapludoll) and Webster (Fine-loamy, mixed, superactive, mesic Typic Endoaquoll) series with less than 3% slope. Sand content ranged from 27% to 53% across the site and clay content ranged from 17% to 32%. In the 5 years prior to sampling, average growing season precipitation at the site was 91.8 cm and mean annual temperature was 48° C. Soil physical properties at each sampling date are reported in Table 4.2.

Soil sampling

In July and October 2012, soils were sampled from the top 10 cm of soil using a 5.5 cm diameter slide-hammer, soil-coring device (Giddings Machine Company, Windsor, CO). Roots for arbuscular mycorrhizal fungi (AMF) colonization counts were collected at the same time using a 2 cm diameter core. Intact soil cores were placed in sterile plastic bags, stored at approximately 4°C, and transported to the laboratory. Five soil and root cores each were collected from every plot at each sampling time. The mass of each individual soil core was used to calculate bulk density. Each soil core was gently broken up along natural points of weakness and passed through an 8 mm sieve (pre-cleaned with ethanol), removing large roots and rocks. Replicated soil cores were combined into one composite sample for each plot. A sub-sample of soil was removed immediately and dried at 105°C for 24 hours to determine field fresh gravimetric water content.

Aggregate fractionation

The remaining soil was prepared for soil aggregate isolation utilizing an "optimal moisture" approach under sterile conditions to standardize soil moisture content and minimize disturbance to microbial communities (Schutter & Dick, 2002; Bach & Hofmockel, 2014). Soil was dried at 4°C in closed, sterile plastic containers with desiccant until soil reached approximately 10% GWC (2-4 days). Approximately five hundred grams of cold-dried soil was placed on a stack of sterile sieves including 2000 μm, 1000 μm, and 250 μm mesh openings. The stack was bolted to a circular sieve shaker intended for soil particle analysis (CSI Scientific). The stack was shaken at approximately 200-250 rpms for three minutes. Soil was gently removed from each sieve and weighed to determine the proportional distribution of mass among soil aggregate fractions. Aggregates fractions are referred to by size: large macroaggregates (>2000 μm), medium macroaggregates (1000-2000 μm), small macroaggregates (250-1000 μm), and microaggregates (<250 μm). Sub-samples of each aggregate fraction and whole soil were frozen immediately at -80 C for subsequent DNA extraction. Aggregate sub-samples were saved to determine post-sieving gravimetric water content, extracellular

enzyme activity, and total C and N. Those data are reported in (Bach & Hofmockel, *in review-b*) and (Williams *et al.*, *in review*).

Soil analyses

As part of a larger study (Bach & Hofmockel, *in review-a*), whole soil sub-samples were used to determine extractable and total soil C and N at the management system scale. Extractable C and N was extracted with 0.5-M potassium sulfate (K_2SO_4) and analyzed by wet-combustion on a TOC-L analyzer (Shimadzu Corporation, Columbia, MD, USA). Total soil C and N was determined by dry combusted in a Thermo Flash 1112 CN analyzer (Thermo Corp, Lakewood, NJ).

Fungal ITS region analysis

DNA was extracted from a 0.25 g sub-sample of soil using the PowerSoil®-htp 96 Well Soil DNA Isolation Kit (MoBio, Carlsbad, CA, USA), following modifications outlined by the Earth Microbiome Project (EMP; www.earthmicrobiome.org/emp-standard-protocols). DNA was quantified via PicoGreen fluorometry and nanodrop. We sequenced the internal transcribed tracer (ITS) region, which best distinguishes fungal lineages (Schoch *et al.*, 2012), following EMP standard protocols (Caporaso *et al.*, 2012). We utilized modified primer set ITS 1F and ITS2 including primers designed to leverage short-read, high depth Illumina MiSeq systems and increase ITS sequences returned (Smith & Peay, 2014). We obtained paired-end 150 base reads from the Illumina MiSeq sequencing system at the Next Generation Sequencing Core (Argonne National Laboratory) and processed the sequence data through the QIIME v.1.7.0 (Caporaso *et al.*, 2010) pipeline using default parameters for quality filtering and demultiplexing. Using QIIME, OTUs were assigned using the UNITE database (Koljalg *et al.*, 2013) and clustering was performed at 97% similarity.

Arbuscular mycorrhizal root colonization

Colonization of root tissue by AMF was determined by the point intercept method. Roots were removed from soil cores (2 cm diameter x 20 cm deep) by washing gently with water over a 53 μm sieve. Cleaned roots were stained with Trypan Blue and stored in a 1:1:1 (vol) solution of glycerin:lactic acid:water at 4°C. Roots were spread in a petri dish marked with 13 mm square grid and examined at 40x magnification to determine presence of fungal structures (hyphae and/or vesicles) at each root-grid line intersection. One hundred intersects were counted for every sample to determine the proportion of root tissue colonized, and each sample was counted twice to ensure reproducible results.

Statistical analysis

All statistical analyses were run in R v.3.1.0 using the vegan package (Oksanen *et al.*, 2013). Before analysis, each sample was rarefied to 1000 sequences to standardize for differences in sequencing depth across samples. Multivariate community analyses were performed on scaled OTU abundances in each sample between 0 and 1 using PERMANOVAs (Anderson, 2001) using the Bray-Curtis distance and 9999 permutations. When comparing differences in community membership, OTU abundances were converted to presence-absence, and PERMANOVAs were performed using Jaccard's distance. Vector analysis was used to correlate environmental and taxanomic groups with community placement using non-metric multidimensional scaling (NMDS). Statistically significant correlations (α=0.05) were included on the NMDS figures with soil C and N variables shown as vectors and taxonomic associations as a labeled point. We evaluated community diversity metrics using richness, evenness, and Shannon's diversity (H). In order to understand how consideration of aggregate fractions in diversity measures compares with standard whole soil measurements, we calculated a proportional whole soil (WSprop), by multiplying the abundance of each unique OTU from a particular aggregate by the proportion that the aggregate makes up of whole soil (sensu Williams *et al.* (*in review*)). Taxa present in multiple aggregate fractions were only counted once in the WSprop sum.

Differences in diversity metrics and abundance of specific taxa were evaluated using linear mixed-effects models using the lme4 package (Bates *et al.*, 2014). Block was considered a random effect and cropping system, sampling date, and soil fraction were the main effects including repeated measures for sampling date and soil fraction. For each variable tested, we used a model selection framework where main effects and interactions were considered (Burnham & Anderson, 2002) and compared with a second-order Akaike's Information Criterion (AIC*c*), allowing for small sample size. Use of AICc accounted for both factor significance and model parsimony with the data (Burnham & Anderson, 2002). We chose the model with significant interaction terms and the lowest AIC_c value by at least 4 points. Differences between least squares means factors within fixed effects were tested for using the difflsmeans function in the lmerTest package in R (Kuznetsova *et al.*, 2014). When considering interactions between main effects (only interactions between cropping system and date were found as significant), we only made comparisons between different cropping systems within dates and similar cropping system across dates. To correct for multiple comparisons across response variables, a Bonferroni correction was used.

Results

Taxonomic distribution

This study is one of the deepest sequencing efforts of soil fungi to date. The rarified fungal dataset included 2,046 unique OTUs from 100,000 sequences. OTU richness ranged from 57 to 354 per sample, which is consistent with richness reported from 454 sequencing studies (Jumpponen *et al.*, 2010; Weber *et al.*, 2013; Smith & Peay, 2014; Voříšková *et al.*, 2014). Unidentified fungal taxa comprised on average 69% of all sequence reads across all samples and did not vary across cropping system or soil aggregate fraction (Table 4.1). Basidiomycota was the most abundant identified fungal phylum comprising 21% of reads. Ascomycota contributed 9% of all reads and Chytridiomycota, Glomeromycota, and Zygomycota each comprised less than 1% of total sequences.

Management system effects

Plant inputs and fertilizer management affected soil C and N pools, which serve as energy and nutrient resources for fungi. Previous research at the COBS site found root inputs were 11 times greater in unfertilized prairie and 9 times greater in fertilized prairie than in continuous maize (Dietzel, 2014). Because all plots were harvested in the fall, the differences in root biomass drove a C input gradient resulting in highest C inputs in unfertilized prairie and lowest C inputs in continuous maize. Coupled with this C input gradient is a contrasting N input gradient. Continuous maize received twice as much inorganic N fertilizer as fertilized prairie and unfertilized prairie received no inorganic N inputs. Total soil C (+ 21%, $F_{2,30}$=4.39, P=0.02) and N (+15%, $F_{2,29}$=4.03, P=0.03) were greater in fertilized prairie than continuous maize (Table 4.2). Total soil C:N ratios did not differ across the management systems in July and October; however, averaged across the entire 2012 growing season (May-Oct), soil C:N was greater in both prairies than in maize (Table 4.2). Extractable C concentrations followed the same pattern as total soil C; however, extractable N was two-fold greater under continuous maize than under either prairie ($F_{2,18}$=10.62, P=0.0009, Table 4.2). Between July and October, extractable C decreased from 39 to 28 μg C g^{-1} dry soil in maize, but increased from 23 to 38 μg C g^{-1} dry soil in fertilized prairie and from 20 to 25 μg C g^{-1} dry soil in unfertilized prairie. Extractable N concentrations did not change between July and October in any system. Plant community, root biomass, and inorganic N inputs played a large role in generating these differences in soil C and N resources across these management systems.

Soil fungal communities responded to both plant inputs and inorganic N inputs at the land-use level. Multivariate analysis (PERMANOVA) of fungal communities indicated interactions between management system and sampling date in both presence/absence (r^2=0.02, P=0.005) and abundance measures (r^2=0.03, P=0.0005). Although all management systems were statistically different from one another based on fungal community composition (Table 4.3), fertilized prairie and maize communities changed between July and October sampling dates and unfertilized prairie fungal communities did not change over time. Across sampling dates, the proportional abundance of Basidiomycota and Ascomycota shifted by in rank abundance where

Basidiomycota were more abundant in July and Ascomycota were more abundant in October. This shift was also mirrored by Chytridomycota, which increased in October. Community richness, evenness, and Shannon's diversity did not differ between management system or sampling date, indicating shifts in specific taxa at the management scale did not affect overall community diversity measures.

Fungal communities in unfertilized prairies contained several taxa not present in the other management systems. Members of the Basidiomycota family Strophariaceae were most abundant in unfertilized prairie ($F_{2,28}$=12.5; P=0.001), and genus *Limonomyces*, which were not present in maize or fertilized prairie, were highly abundant in unfertilized prairie ($F_{2,29}$=4.5; P=0.02, Fig. 4.1b,c, Table 4.2). Furthermore, the abundance of *Limonomyces* drove the positive correlation of chitinase (N-acetyl glucosaminidase) enzyme activity with unfertilized prairie communities (r^2=0.10, P=0.01, Fig. 4.1c). All other extracellular enzyme activities were more strongly correlated with soil C and N pools than any specific fungal taxa.

Fungal communities in fertilized prairie shared more taxa in common with unfertilized prairie. Members of the Basidiomycota genus *Thanatephorus* were not present in maize and present in ten fertilized prairie samples compared with only two samples unfertilized prairie ($F_{1,11}$=44.3; P<0.0001, Table 4.2). Psathyrellaceae was four times more abundant in fertilized prairie than in unfertilized prairie and mean abundance in maize was intermediary ($F_{2,26}$=3.23; P=0.05; Fig. 4.1c, Table 4.2). Within the phyla Ascomycota, fertilized prairie supported greater abundance of the family Orbiliaceae ($F_{2,68}$=8.3; P=0.0005) and presence of *Trichoderma citrinoviride* ($F_{2,59}$=1.8; P=0.04) than the other management systems (Table 4.2).

Compared to perennial prairies, fungal communities in annual continuous maize were characterized by greater abundance of unknown Basidiomycota taxa ($F_{2,114}$=8.8; P=0.001) and presence of the Glomeromycota order Glomerales ($F_{2,32}$=3.0; P=0.03, Fig. 4.1b,c; Table 4.2). Unknown sequences most similar to Basidiomycota were among the most abundant taxa across all systems. In maize soils, the lower abundance of other taxa enhanced the rank abundance of these unknown Basidiomycota. In contrast, Glomerales had low abundance in all management systems, and its increased relative presence drove this order's importance in maize more so than absolute abundance. Counts of root

arbuscular mycorrhizal colonization (AMF) indicated that because maize had significantly lower root biomass, individual roots had 6-10 times greater percentage of root colonization than the either prairie ($F_{2,20}$=59.0; P<0.0001, Fig. 4.2). Interestingly, root colonization counts found greater proportion of vesicle presence in prairie compared with maize, which had much greater hyphal presence ($F_{2,24}$=101.9; P<0.0001, Fig. 4.2). The abundance of taxa from the Ascomycota phyla was two-fold greater in maize and fertilized prairie than unfertilized prairie ($F_{2,432}$=4.5; P=0.01, Table 4.2) and 78% greater in October than in July. These responses were largely driven by taxa from the genus *Peziza,* which were only found in fertilized prairies and maize and were more abundant in October than July ($F_{1,23}$=3.9; P=0.05).

Soil aggregate fractions

We observed changes in fungal community structure and diversity across aggregate fractions. Large macroaggregates (>2000 μm) supported different fungal communities than microaggregates (<250 μm) (R^2=0.06, P=0.005, Fig. 4.1a). This was driven by increased fungal richness (F_5=21, P<0.0001) and Shannon's diversity (H) (F_5=6.3, P<0.0001) in microaggregate fractions (Fig. 4.3). Presence of the Basidiomycota family Strophariaceae was positively correlated with increasing aggregate fraction (r^2=0.20; P=0.02).

These differences in fungal community composition at the soil aggregate scale can be masked in whole soil sampling approaches. We reconstructed a proportional whole soil measure of fungal richness, diversity and evenness by calculating a weighted sum of these measures from each aggregate fraction that accounts for the mass distribution of each aggregate fraction within the whole soil (sensu Williams *et al.* (*in review*)). Calculated proportional whole soil had two-fold greater richness and 20% greater Shannon's diversity than measured whole soil samples (F_5=6.3, P<0.001, Fig. 4.3). By mass, free microaggregates contribute less than 7% to whole soil mass, but they contain 65% of total OTUs detected, including 92 OTUs not found in any other soil fraction. This underscores how much the presence of rare taxa in microaggregates can

contribute to soil fungal diversity and how that diversity may not be captured with traditional whole soil sampling techniques (Williams *et al.*, *in review*).

Total C and N resources also varied at the micro-scale, between soil aggregate fractions (Bach & Hofmockel, *in review-b;* Williams *et al.*, *in review*). At these two sampling dates, large and medium macroaggregates had 20% more TC than microaggregates (Williams *et al.*, *in review*). Total N followed a similar pattern, but with less magnitude of difference between the fractions. As a result, total C:N ratios within soil aggregates was greatest in large and medium macroaggregates and least in microaggregates. These results are consistent with the paradigm of large macroaggregates containing more fresh plant inputs, which have a wider C:N ratio signature than microbially-processed organic matter, which is predominant in micoraggregate fractions (Grandy & Neff, 2008; Hofmockel *et al.*, 2011). Fungi contribute toward this processing of organic matter and respond to changes in substrate quantity and quality.

Discussion

Fungal communities responded to differences in C and N resources at both the macro-scale (m), between management systems, and the micro-scale (μm), among soil aggregates. Supporting hypothesis 1, distinct fungal communities had developed in continuous maize, unfertilized prairie, and fertilized prairie after four years of management. Fungal communities in maize and fertilized prairie shifted between July and October sampling dates, but remained constant in unfertilized prairies. In addition, deep sequencing of fungal communities at the soil aggregate scale found whole soil sampling approaches can underestimate fungal richness and diversity, supporting hypothesis 2.

Fungal community structure responded to differences in soil total soil C and N between management systems. Increased complex, plant-derived, organic matter and the absence of inorganic nutrient inputs in unfertilized prairie led to increased presence of Basidiomycota , such as Strophariaceae and *Limonomyces*. Strophariaceae is a large family including 18 genera and more than 1300 species, including many taxa with a high

capacity for ligno-cellulolytic decomposition, which would have a competitive advantage with increasing root litter inputs (Lynd *et al.*, 2002; Osono, 2007; Tian *et al.*, 2010). Unfertilized prairie roots have a lignin/N ratio of 30 compared to 15 for fertilized prairie roots (Rivas *et al.*, 2014), and these low quality substrates likely contribute to the competitive advantage of Strophariaceae. These lignin/N ratios are lower than values reported in forest litter (St. John *et al.*, 2011), and lignin degradation has not traditionally been considered a selecting force in grassland and agrecosystem fungal community structure. Alternatively, inorganic fertilizer may negatively impact Strophariaceae, as has been shown for other Basidiomycota taxa (Nemergut *et al.*, 2008; Allison *et al.*, 2010; Weber *et al.*, 2013). *Limonomyces* are known to cause "pink disease" in turf grass (Stalpers & Loerakker, 1982), and the importance of *Limonomyces* in unfertilized prairie communities and its correlation with chitinase activity forms several compelling hypotheses about potential organic N cycling and further ecological investigation of this lineage is merited.

Within fertilized prairies, greater total soil C and N shaped fungal communities characterized by members of the Basidiomycota family Psathyrellaceae, genus *Thanatephorus, Trichodema citrinoviride*, and Ascomycota family Orbiliacea. Soil C and N resources result from direct inputs of inorganic N fertilizer in the early spring and increased abundance of cool-season (C_3 photosynthesis) plants in fertilized prairies (Jarchow & Liebman, 2013), which produce roots with lower C:N ratios than roots in unfertilized prairie systems (Jarchow & Liebman, 2012). All of the aforementioned fungal lineages include ligno-cellulose degraders and taxa that grow readily on labile C substrates, and these fertilized prairie roots have cellulose (103 g kg^{-1} soil) and lignin (169 g kg^{-1} soil) concentrations lower than unfertilized prairie and maize roots (Rivas *et al.*, 2014). However, there is not enough known about specific ecological functioning of these taxa to draw conclusions about their role in processing plant inputs that may drive increases in soil C and N, but these taxa would be strong candidates to focus future research on metabolic functioning.

We observed further reason for caution in interpreting fungal taxonomy without strong ecological context in maize management systems. High abundance of Basidiomycota, particularly unidentified taxa in maize runs counter to the classic

association of Basidiomycota with degradation of complex C substrates, such a lignin, and organic N (Boddy & Jones, 2006). In our study, maize soils had twice as much extractable N and similar levels of extractable C than either prairie, but lower total N and total C. In forest systems, inorganic N addition has been shown to decrease the abundance of Basidiomycota (Nemergut *et al.*, 2008; Allison *et al.*, 2010; Weber *et al.*, 2013). Thus, these unknown Basidiomycota taxa are responding counter to previously observed phyla-level shifts, underscoring how little we understand about these fungi in agricultural systems.

Arbuscular mycorrhizal fungi in the order Glomerales were also associated with maize systems, supporting microscopically observed AMF root colonization rates. Increased presence of Glomerales OTUs within maize coincided with increased rates of root colonization, specifically hyphal colonization. The family Glomaceae within Glomerales are rapid root colonizers and have greater presence within root tissue than other AMF lineages (Hart & Reader, 2002) , and spore studies have shown that a few taxa within Glomerales, including *Glomus intraradices* and *Glomus diaphanum*, dominate crop land (Oehl *et al.*, 2010). The rapid colonization capabilities of Glomaceae may be an advantage in annual maize, whereas slower-colonizing, but greater plant return on investment, may give other AMF lineages an advantage in perennial prairie systems, driving greater AMF diversity in grasslands (Verbruggen *et al.*, 2010). It remains unclear from our dataset if increased presence of Glomerales may be contributing the more than two-fold lower rate of vesicle colonization observed in maize roots. There is a family, Gigasporaceae, in the order Diversisporales that does not produce vesicles (Oehl *et al.*, 2011; Redecker *et al.*, 2013), but we did not observe any Gigasporaceae OTUs in our samples.

In our data set, the abundance of Glomeromycota taxa was very low overall, indicating they are a minor contribution to the total fungal community and/or sequencing approaches from soil samples may underrepresent these plant symbionts. Our data are consistent with previous works reporting 1% sequences belonging to the phyla Glomeromycota in tallgrass prairie (Jumpponen *et al.*, 2010) and 2-5% in forest systems (Kerekes *et al.*, 2013; Weber *et al.*, 2013; Smith & Peay, 2014; Voříšková *et al.*, 2014). However, our observed 90% AMF colonization rates on maize roots indicates these fungi

are abundant in root tissue, and a large body of literature has shown AMF play a critical role in crop production (Verbruggen & Toby Kiers, 2010; Verbruggen *et al.*, 2013) and grassland plant community interactions (Hartnett & Wilson, 1999; Dumbrell *et al.*, 2011; Hiiesalu *et al.*, 2014). It is possible that DNA extraction from aggregates in bulk soil under-represents the presence of these fungi, which are obligate plant symbionts. In addition, ITS primers have been shown to bias toward Basidiomycota and Ascomycota (Bellemain *et al.*, 2010), meaning they may be less effective at binding and replicating ITS regions in basal fungal taxa, including Glomeromycota. Within Glomeromycota, the ITS region does a poor job distinguishing taxa at the species level (Stockinger *et al.*, 2009). The UNITE database also relies on complete or nearly complete ITS sequences and sequences from cultured or sporocarp samples are given preference for taxonomic reference (Koljalg *et al.*, 2013), which ensures the most accurate match between sequence and taxa, but may also leave uncultured and symbiotic fungi, including Glomeromycota, underrepresented.

At the micro-scale, C and N resources contributed to differences in fungal community richness and diversity between soil aggregate fractions. Greater total C and N resources in large macroaggregates, appears to facilitate dominance of the fungal community by a few members, namely the Basidiomycota family Strophariceae. As mentioned above, Strophariaceae includes many taxa involved in ligno-cellulolytic decomposition (Lynd *et al.*, 2002; Osono, 2007; Tian *et al.*, 2010), which supports the hypothesis that large macroaggregates containing more plant-derived organic matter. Lower total C and N, and a lower C:N ratio indicative of more microbially-processed organic matter in microaggregates (Six *et al.*, 2000a; Grandy & Neff, 2008), increased fungal community richness. Increased taxonomic richness in microaggregates may be due to fungi stabilized on the surface of these free microaggregates where fungi largely interact with organic matter in surrounding pore space. Increased bacterial community richness was also observed in these same microaggregates (Williams *et al.*, *in review*). In contrast to the bacterial communities, the increase in fungal richness in microaggregates was sufficient to cause an increase in overall fungal diversity in microaggregates compared to other fractions. This is driven in part by lower numbers of fungal taxa, an order of magnitude less than the number of bacterial taxa observed. In both the bacterial

study and this one, traditional whole soil sampling approaches underestimated soil microbial community richness calculated from the proportional sum of all aggregate fraction richness measures. Thus, for hypotheses focused on capturing rare members of the soil community, including microorganisms with potentially unrecognized metabolic functions, consideration of the microaggregate fraction may provide important insight.

This unprecedented deep sequencing of fungal communities in managed grasslands and agricultural systems provides several key insights into understanding the distribution of fungi across management systems and within soil. In congruence with previous work, we found fungal communities were strongly shaped by ecosystem management and plant inputs. We were able to identify shifts in fungal communities as C and N resources varied in availability across three management systems in the same soil. In addition, we saw changes in soil fungal community richness and diversity across soil aggregate fractions. Fungal richness increased with decreasing total C, N, and C:N ratio in microaggregate fractions. Together, these results show fungal communities responding to changes in C and N quantity and quality at both macro- and micro-scales. Identifying and understanding macro- and micro-scale drivers of fungal community structure is crucial to understanding biological processes and feedbacks above- and belowground. This is especially true in agricultural systems where fungi can range from devastating crop pathogens to beneficial anti-pathogens and nutrient cyclers.

Acknowledgements

Soil collection and processing was funded by a USDA-NASA grant (K.S.H.). Sequencing was funded by the Leopold Center for Sustainable Agriculture (K.S.H. and E.M.B) and Lois F. Tiffany Award from Iowa State University (E.M.B.). Additional sequencing technical support provided by DOE Genomic Sciences and Sarah Owens at Argonne National Laboratory. Giselle Narvaez and Kira Murray collected the AMF root colonization data. Rebecca Luzbetak, Sarah Hargreaves, Aaron Sharp, and Fang Yang provided valuable field, laboratory, bioinfomatic, and editorial assistance.

Literature Cited

Allison SD, Gartner TB, Mack MC, McGuire K, Treseder K. 2010. Nitrogen alters carbon dynamics during early succession in boreal forest. Soil Biology and Biochemistry 42(7): 1157-1164.

Anderson MJ. 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26(1): 32-46.

Angers DA, Recous S, Aita C. 1997. Fate of carbon and nitrogen in water-stable aggregates during decomposition of (CN)-C-13-N-15-labelled wheat straw in situ. European Journal of Soil Science 48(2): 295-300.

Appuhn A, Joergensen RG. 2006. Microbial colonisation of roots as a function of plant species. Soil Biology and Biochemistry 38(5): 1040-1051.

Bach EM, Hofmockel K. in review-a. Coupled Carbon and Nitrogen inputs increase microbial biomass and activity in prairie bioenergy systems. Ecosystems.

Bach EM, Hofmockel K. in review-b. Soil structure and seasonality affect microbial activity in managed grasslands. Ecosphere.

Bach EM, Hofmockel KS. 2014. Soil aggregate isolation method affects measures of intra-aggregate extracellular enzyme activity. Soil Biology & Biochemistry 69(0): 54-62.

Bates D, Maechles M, Bolker B, Walker S 2014. lme4: Linear mixed-effects models using Eigen and S4: R package version 1.0-6.

Bellemain E, Carlsen T, Brochmann C, Coissac E, Taberlet P, Kauserud H. 2010. ITS as an environmental DNA barcode for fungi: an in silico approach reveals potential PCR biases. Bmc Microbiology 10.

Boddy L, Jones TH 2006. Mycelial responses in heterogeneous environments: Parallels with macroorganisms. In: Gadd GM, Watkinson SC, Dyer PS eds. Fungi in the Environment. Cambridge, UK: Cambridge University Press, 112-140.

Broeckling CD, Broz AK, Bergelson J, Manter DK, Vivanco JM. 2008. Root Exudates Regulate Soil Fungal Community Composition and Diversity. Applied and Environmental Microbiology 74(3): 738-744.

Burnham KP, Anderson DR. 2002. Model Selection and Multimodel Inference. New Jersey, USA: Springer.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, et al. 2010. QIIME allows analysis of high-throughput community sequencing data. Nat Meth 7(5): 335-336.

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, Owens SM, Betley J, Fraser L, Bauer M, et al. 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J 6(8): 1621-1624.

Denef K, Six J, Bossuyt H, Frey SD, Elliott ET, Merckx R, Paustian K. 2001. Influence of dry-wet cycles on the interrelationship between aggregate, particulate organic matter, and microbial community dynamics. Soil Biology & Biochemistry 33(12-13): 1599-1611.

Dietzel R. 2014. Comparing soil carbon storage potential in corn- and prairie-based cropping systems. Ph. D., Iowa State Univeristy.

Drazkiewicz M. 1996. Generic composition of fungi in soil aggregates. Folia Microbiologica 41(3): 272-276.

Dumbrell AJ, Ashton PD, Aziz N, Feng G, Nelson M, Dytham C, Fitter AH, Helgason T. 2011. Distinct seasonal assemblages of arbuscular mycorrhizal fungi revealed by massively parallel pyrosequencing. New Phytologist 190(3): 794-804.

Ekelund F, Ronn R, Christensen S. 2001. Distribution with depth of protozoa, bacteria and fungi in soil profiles from three Danish forest sites. Soil Biology & Biochemistry 33(4-5): 475-481.

Fierer N, Breitbart M, Nulton J, Salamon P, Lozupone C, Jones R, Robeson M, Edwards RA, Felts B, Rayhawk S, et al. 2007. Metagenomic and small-subunit rRNA analyses reveal the genetic diversity of bacteria, archaea, fungi, and viruses in soil. Applied and Environmental Microbiology 73(21): 7059-7066.

Grandy AS, Neff JC. 2008. Molecular C dynamics downstream: The biochemical decomposition sequence and its impact on soil organic matter structure and function. Science of the Total Environment 404(2-3): 297-307.

Guggenberger G, Christensen BT, Zech W. 1994. Land-use effects on the composition of orgainc-matter in particle-size separates of soil.1. Lignin and carbohydrate signature. European Journal of Soil Science 45(4): 449-458.

Hart MM, Reader RJ. 2002. Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. New Phytologist 153(2): 335-344.

Hartnett DC, Hetrick BAD, Wilson GWT, Gibson DJ. 1993. Mycorrhizal Influence on Intra- and Interspecific Neighbour Interactions among Co-Occurring Prairie Grasses. Journal of Ecology 81(4): 787-795.

Hartnett DC, Wilson GWT. 1999. Mycorrhizae influence plant community structure and diversity in tallgrass prairie. Ecology 80(4): 1187-1195.

Hendrix PF, Parmelee RW, Crossley DA, Jr., Coleman DC, Odum EP, Groffman PM. 1986. Detritus Food Webs in Conventional and No-Tillage Agroecosystems. BioScience 36(6): 374-380.

Hiiesalu I, Pärtel M, Davison J, Gerhold P, Metsis M, Moora M, Öpik M, Vasar M, Zobel M, Wilson SD. 2014. Species richness of arbuscular mycorrhizal fungi: associations with grassland plant richness and biomass. New Phytologist: n/a-n/a.

Hofmockel KS, Zak DR, Moran KK, Jastrow JD. 2011. Changes in forest soil organic matter pools after a decade of elevated CO2 and O3. Soil Biology and Biochemistry 43(7): 1518-1527.

Jarchow ME, Liebman M. 2012. Tradeoffs in biomass and nutrient allocation in prairies and corn managed for bioenergy production. Crop Science 52(3): 1330-1342.

Jarchow ME, Liebman M. 2013. Nitrogen fertilization increases diversity and productivity of prairie communities used for bioenergy. GCB Bioenergy 5(3): 281-289.

Jastrow JD. 1996. Soil aggregate formation and the accrual of particulate and mineral-associated organic matter. Soil Biology & Biochemistry 28(4-5): 665-676.

Joergensen RG, Wichern F. 2008. Quantitative assessment of the fungal contribution to microbial tissue in soil. Soil Biology and Biochemistry 40(12): 2977-2991.

Jumpponen A, Jones KL, Blair J. 2010. Vertical distribution of fungal communities in tallgrass prairie soil. Mycologia 102(5): 1027-1041.

Karst J, Piculell B, Brigham C, Booth M, Hoeksema JD. 2013. Fungal communities in soils along a vegetative ecotone. Mycologia 105(1): 61-70.

Kerekes J, Kaspari M, Stevenson B, Nilsson RH, Hartmann M, Amend A, Bruns TD. 2013. Nutrient enrichment increased species richness of leaf litter fungal assemblages in a tropical forest. Molecular Ecology 22(10): 2827-2838.

Koljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AF, Bahram M, Bates ST, Bruns TD, Bengtsson-Palme J, Callaghan TM, et al. 2013. Towards a unified paradigm for sequence-based identification of fungi. Mol Ecol 22(21): 5271-5277.

Kuznetsova A, Brockhoff PB, Christensen RHB 2014. lmerTest: Tests for random and fixed effects for linear mixed effect models (lmer objects of lme4 package). R package version 2.0-6.

Lynd LR, Weimer PJ, van Zyl WH, Pretorius IS. 2002. Microbial Cellulose Utilization: Fundamentals and Biotechnology. Microbiology and Molecular Biology Reviews 66(3): 506-577.

Nemergut DR, Townsend AR, Sattin SR, Freeman KR, Fierer N, Neff JC, Bowman WD, Schadt CW, Weintraub MN, Schmidt SK. 2008. The effects of chronic nitrogen fertilization on alpine tundra soil microbial communities: implications for carbon and nitrogen cycling. Environmental Microbiology 10(11): 3093-3105.

Oehl F, Alves a Silva G, Goto BT, Costa Maia L, Sieverding E. 2011. Glomeromycota: two new classes and a new order. Mycotaxon 116(1): 365-379.

Oehl F, Laczko E, Bogenrieder A, Stahr K, Bösch R, van der Heijden M, Sieverding E. 2010. Soil type and land use intensity determine the composition of arbuscular mycorrhizal fungal communities. Soil Biology and Biochemistry 42(5): 724-738.

Oksanen J, Blanchet FG, Kindt R, Legandre P, Minchin PR, O'Hara RB 2013. vegan: Community ecology package: R package version 2.0-1.0.

Osono T. 2007. Ecology of ligninolytic fungi associated with leaf litter decomposition. Ecological Research 22(6): 955-974.

Peay KG, Baraloto C, Fine PVA. 2013. Strong coupling of plant and fungal community structure across western Amazonian rainforests. ISME J 7(9): 1852-1861.

Plante AF, McGill WB. 2002. Intraseasonal soil macroaggregate dynamics in two contrasting field soils using labeled tracer spheres Soil Science Society of America Journal 66(4): 1285-1295.

Redecker D, Schüßler A, Stockinger H, Stürmer S, Morton J, Walker C. 2013. An evidence-based consensus for the classification of arbuscular mycorrhizal fungi (Glomeromycota). Mycorrhiza 23(7): 515-531.

Rillig MC, Mummey DL. 2006. Mycorrhizas and soil structure. New Phytologist 171(1): 41-53.

Rivas FA, Tabatabai MA, Olk DC, Thompson ML. 2014. Kinetics of short-term carbon mineralization in roots of biofuel crops in soils. Biology and Fertility of Soils 50(3): 527-535.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Consortium FB. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proceedings of the National Academy of Sciences 109(16): 6241-6246.

Schutter ME, Dick RP. 2002. Microbial community profiles and activities among aggregates of winter fallow and cover-cropped soil. Soil Science Society of America Journal 66(1): 142-153.

Six J, Elliott ET, Paustian K. 2000. Soil macroaggregate turnover and microaggregate formation: A mechanism for C sequestration under no-tillage agriculture. Soil Biology & Biochemistry 32: 2099-2103.

Six J, Frey SD, Thiet RK, Batten KM. 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Science Society of America Journal 70(2): 555-569.

Smith DP, Peay KG. 2014. Sequence Depth, Not PCR Replication, Improves Ecological Inference from Next Generation DNA Sequencing. PLoS ONE 9(2): e90234.

St. John MG, Orwin KH, Dickie IA. 2011. No 'home' versus 'away' effects of decomposition found in a grassland–forest reciprocal litter transplant study. Soil Biology and Biochemistry 43(7): 1482-1489.

Stalpers JA, Loerakker WM. 1982. Laetisaria and Limonomyces species (Corticiaceae) causing pink diseases in turf grasses. Canadian Journal of Botany 60(5): 529-537.

Stockinger H, Walker C, Schüßler A. 2009. 'Glomus intraradices DAOM197198', a model fungus in arbuscular mycorrhiza research, is not Glomus intraradices. New Phytologist 183(4): 1176-1187.

Strickland MS, Rousk J. 2010. Considering fungal:bacterial dominance in soils â€“ Methods, controls, and ecosystem implications. Soil Biology and Biochemistry 42(9): 1385-1395.

Talbot JM, Bruns TD, Taylor JW, Smith DP, Branco S, Glassman SI, Erlandson S, Vilgalys R, Liao H-L, Smith ME, et al. 2014. Endemism and functional convergence across the North American soil mycobiome. Proceedings of the National Academy of Sciences.

Tian B-Y, Huang Q-G, Xu Y, Wang C-X, Lv R-R, Huang J-Z. 2010. Microbial community structure and diversity in a native forest wood-decomposed hollow-stump ecosystem. World Journal of Microbiology and Biotechnology 26(2): 233-240.

Tisdall JM, Oades JM. 1982. Organic-matter and water-stable aggregates in soils. Journal of Soil Science 33(2): 141-163.

Verbruggen E, Röling WFM, Gamper HA, Kowalchuk GA, Verhoef HA, van der Heijden MGA. 2010. Positive effects of organic farming on below-ground mutualists: large-scale comparison of mycorrhizal fungal communities in agricultural soils. New Phytologist 186(4): 968-979.

Verbruggen E, Toby Kiers E. 2010. Evolutionary ecology of mycorrhizal functional diversity in agricultural systems. Evolutionary Applications 3(5-6): 547-560.

Verbruggen E, van der Heijden MGA, Rillig MC, Kiers ET. 2013. Mycorrhizal fungal establishment in agricultural soils: factors determining inoculation success. New Phytologist 197(4): 1104-1109.

Voříšková J, Brabcová V, Cajthaml T, Baldrian P. 2014. Seasonal dynamics of fungal communities in a temperate oak forest soil. New Phytologist 201(1): 269-278.

Weber CF, Vilgalys R, Kuske CR. 2013. Changes in fungal community composition in response to elevated atmospheric CO2 and nitrogen fertilization varies with soil horizon. Frontiers in Microbiology 4.

Williams RJ, Bach EM, Howe A, Hofmockel K. in review. Soil Aggregates Foster Under-sampled Microbial Communities that are Structured Through Carbon and Nitrogen Limitation. ISME J.

Tables

Table 4.1: Proportion (± 1 SE) of total sequence reads assigned to fungal phyla and unidentified sequences in maize, prairie, and fertilized prairie. Different letter denote statistically difference between the cropping systems (α=0.05).

Crop	Unknown	Asco-mycota	Basidio-mycota	Chytridio-mycota	Glomero-mycota	Zygo-mycota
CC	0.657± 0.005	0.079± 0.003^{B}	0.258± 0.006^{A}	2.6E± 0.3E-4	3.2± 0.2E-4	4.4± 0.3E-3
P	0.726± 0.005	0.042± 0.001^{C}	0.230± 0.006^{AB}	8.5± 0.3E-4	1.8± 0.2E-4	1.4± 0.5E-3
PF	0.698± 0.004	0.132± 0.002^{A}	0.165± 0.004^{B}	6.7± 0.3E-4	8.3± 0.1E-4	4.8± 0.2E-3
All	0.694± 0.002	0.086± 0.0008	0.215± 0.002	6.0± 0.1E-4	1.9± 0.05E-4	3.39± 0.07E-3

Table 4.2: Soil C and N pools from whole soil and sequence abundance of fungal taxa across management systems: unfertilized prairie, fertilized prairie, and continuous maize (n=12). Mean values are presented, ± 1 SE. Statistically different values between management systems indicated by different letter (α=0.05). For fungal taxa, the number of samples in which the OTU was detected is presented in parentheses, as in some cases rate of presence of taxa differed, but not mean abundance. NP=not present.

	Unfertilized Prairie	**Fertilized Prairie**	**Continuous Maize**
Total soil C* (g C Kg^{-1} soil)	24.4±2.2	26.7±2.2	21.9±2.2
Total soil N* (g N Kg^{-1} soil)	1.9±0.1	2.0±0.1	1.7±0.1
Total C:N ratio*	12.9±0.2	13.2±0.2	12.8±0.2
Extractable soil C* (µg C g-1 dry soil)	22.8±5.4	30.9±5.0	33.7±5.0
Extractable soil N* (µg N g-1 dry soil)	$4.3±0.8^{B}$	$5.1±0.8^{B}$	$9.4±0.8^{A}$
Basidiomycota	43.2±7.7	26.2±3.6	51.9±7.8
Strophariaceae	235±71 (12)	58±22 (14)	24±8 (6)
Psathyrellaceae	7±5 (4)	31±8 (20)	16 (1)
Limonomyces	78±21 (18)	NP	NP
Thanatephorus	24.5±0.7 (2)	31±11 (10)	NP
Unidentified Basidiomycota	3.0±0.9 (16)	18±4 (10)	54±24 (16)
Ascomycota	11.5±1.6	23±3	23±4
Orbiliaceae	17±5 (22)	67±12 (41)	2.7±1.8 (6)
Peziza	NP	32±9 (16)	23±11 (5)
Trichoderma citrinoviride	11±2 (15)	17±2 (30)	21±3 (18)
Glomeromycota (AMF)	1.5±0.3 (4)	1.2±0.2 (6)	1.0 (10 samples, value=1 for all)
Glomerales	NP	1.2±0.2 (5)	1.0 (10 samples, value=1 for all)
Zygomycota	2.7±0.8 (15)	5.6±1.3 (30)	10.8±3.2 (13)
Chytridiomycota	1.5±0.2 (13)	1.4±0.2 (18)	1.3±0.2 (10)

Subset of data presented in Bach & Hofmockel *in review

Table 4.3: PERMANOVA Pairwise comparisons of fungal communities between cropping systems at July and October sampling dates. Bonferroni corrected α=0.005.

Comparison	**R^2**	**P**	**Full Model P**
July			*0.0001*
P-PF	0.08	0.0002	
CC-P	0.18	0.0001	
CC-PF	0.17	0.0001	
Oct			*0.0001*
P-PF	0.07	0.0001	
CC-P	0.10	0.0001	
CC-P	0.09	0.0001	
P: July-Oct	0.05	0.06	
PF: July-Oct	0.06	0.0002	
CC: July-Oct	0.06	0.002	

Figures

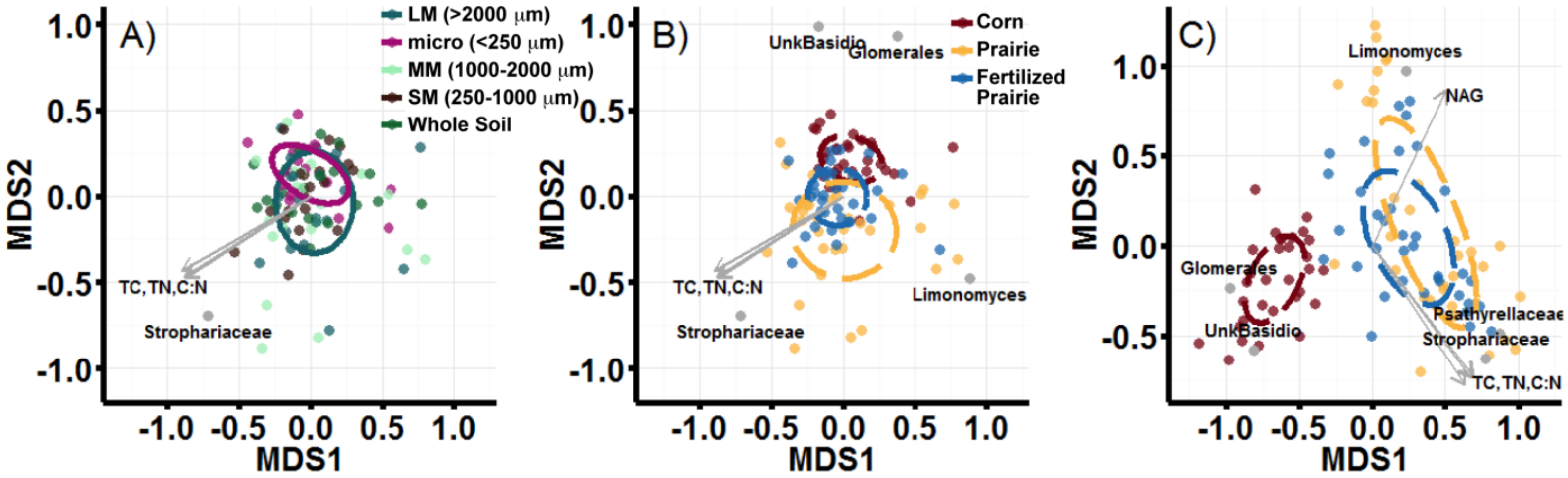

Figure 4.1: Non-metric multidimensional scaling (NMDS) of presence/absence of fungal taxa in soil aggregate fractions (A) from maize, unfertilized prairie, and fertilized prairie ecosystems (B). Abundance of fungal taxa also differed between the three cropping systems (C). Taxa statistically correlated with community types are shown by gray points. Statistically correlated environmental variables are shown as gray arrows (α=0.05).

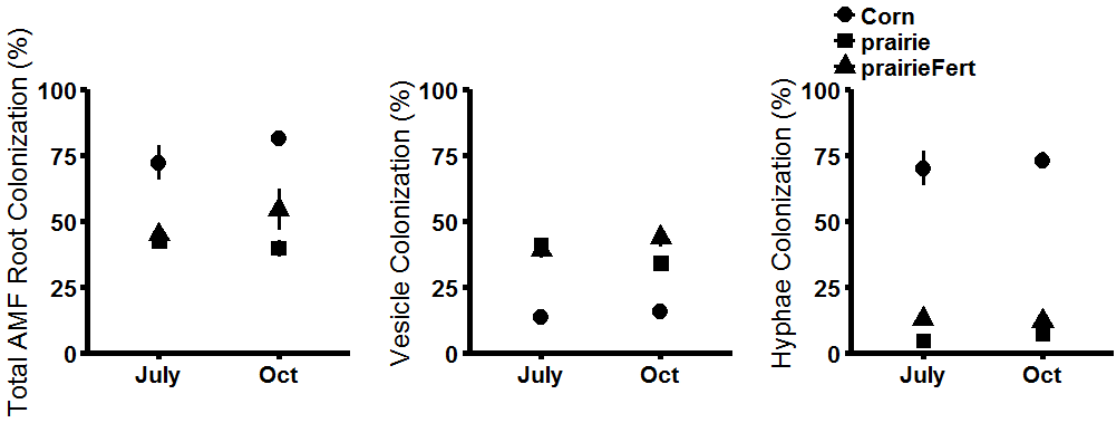

Figure 4.2: Arbuscular mycorrhizal fungi (AMF; Glomeromycota) A) total root colonization rate, B) vesicle colonization rate, and C) hyphal colonization rate in maize, prairie, and fertilized prairie systems in July and October 2012.

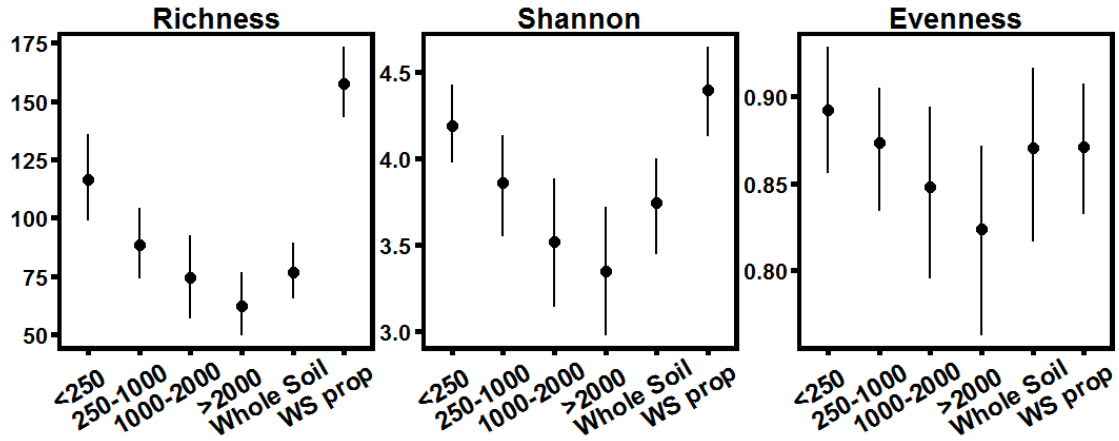

Figure 4.3: Fungal community A) richness, B) Shannon's diversity, and C) evenness among soil aggregate fractions and proportional whole soil (WSprop), a weighted sum of the metric measured in aggregate fractions (ɣ diversity).

Supplemental Information

Table 4.4S: Total sequences of most abundant fungal taxa by phyla, family, and species in unfertilized prairie, fertilize prairie, and maize bioenergy systems.

	Unfertilized Prairie		**Fertilized Prairie**		**Continuous Maize**	
Phyla						
	unk	24018	unk	24018	unk	20081
	Basidiomycota	7569	Basidiomycota	7569	Basidiomycota	8262
	Ascomycota	1354	Ascomycota	1354	Ascomycota	2493
	Zygomycota	39	Zygomycota	39	Zygomycota	145
	Chytridiomycota	14	Chytridiomycota	14	Chytridiomycota	10
	Glomeromycota	6	Glomeromycota	6	Glomeromycota	9
Family						
	Strophariaceae	2800	unidentified	2734	Entolomataceae	1524
	Entolomataceae	1446	Entolomataceae	1217	Bolbitiaceae	432
	Corticiaceae	1396	Marasmiaceae	1006	Hypocreaceae	419
	unidentified	459	Strophariaceae	850	Agaricaceae	399
	Agaricaceae	273	Psathyrellaceae	611	Stephanosporaceae	167
	Bolbitiaceae	228	Incertae sedis	550	Incertae sedis	159
	Hypocreaceae	169	Hypocreaceae	497	Strophariaceae	154
	Atheliaceae	135	Pezizaceae	489	Typhulaceae	147
	Incertae sedis	117	Agaricaceae	398	Pleosporaceae	134
Species						
	Limonomyces sp	1396	Orbiliomycetes sp	2729	*Trichoderma citrinoviride*	419
	Orbiliomycetes sp	433	*Marasmius capillaris*	962	*Conocybe apala*	403
	Trichoderma citrinoviride	169	*Trichoderma citrinoviride*	497	*Clitopilus sp*	338
	Athelia bombacina	135	*Leptosphaerulina sp*	395	*Vascellum spNY_2013a*	180
	Vascellum spNY_2013a	132	*Peziza varia*	360	*Cristinia helvetica*	167
	Lycoperdon pyriforme	123	*Calvatia gigantea*	197	*Leptosphaerulina sp*	140
	Leptosphaerulina sp	75	*Pholiotina dentatomarginata*	164	*Drechslera spBAFC3419*	133
	Typhula maritima	66	*Sphaerobolus sp*	84	*Typhula maritima*	133
	Basidiomycota sp	26	*Scolecobasidium humicola*	60	*Agrocybe pediades*	91

Table 4.5S: Total sequences of most abundant fungal taxa by phyla, family, and species across soil aggregate fractions (LM >2000 µm, MM 100-2000 µm, SM 250-1000 µm, Micro <250 µm, and WS whole soil).

	LM		MM		SM		Micro		WS	
Phyla										
	unk	15559	unk	14384	unk	14107	unk	10337	unk	14797
	Basidiomycota	4020	Basidiomycota	4764	Basidiomycota	4884	Basidiomycota	3518	Basidiomycota	4556
	Ascomycota	1349	Ascomycota	1799	Ascomycota	1914	Ascomycota	2012	Ascomycota	1574
	Zygomycota	50	Zygomycota	42	Zygomycota	85	Zygomycota	116	Zygomycota	58
	Chytridiomycota	15	Chytridiomycota	10	Chytridiomycota	7	Chytridiomycota	16	Chytridiomycota	8
	Glomeromycota	7	Glomeromycota	1	Glomeromycota	3	Glomeromycota	1	Glomeromycota	7
Family										
	Entolomataceae	1461	unidentified	877	Strophariaceae	1284	Strophariaceae	879	Entolomataceae	860
	Strophariaceae	490	Entolomataceae	776	unidentified	701	unidentified	620	unidentified	597
	unidentified	419	Strophariaceae	728	Entolomataceae	593	Entolomataceae	497	Strophariaceae	423
	Corticiaceae	297	Marasmiaceae	718	Bolbitiaceae	415	Corticiaceae	352	Agaricaceae	290
	Agaricaceae	261	Psathyrellaceae	290	Corticiaceae	321	Incertaesedis	338	Corticiaceae	278
	Hypocreaceae	240	Agaricaceae	200	Hypocreaceae	265	Hypocreaceae	237	Bolbitiaceae	175
	Ceratobasid-iaceae	221	Bolbitiaceae	198	Agaricaceae	232	Typhulaceae	150	Psathyrellaceae	172
	Pezizaceae	195	Hypocreaceae	197	Marasmiaceae	129	Pezizaceae	101	Hypocreaceae	146
	Bolbitiaceae	181	Pezizaceae	184	Incertae sedis	128	Psathyrellaceae	93	Incertae sedis	138
Species										
	Orbiliomycetes sp	418	*Orbiliomycetes sp*	873	*Orbiliomycetes sp*	697	*Orbiliomycetes sp*	598	*Orbiliomycetes sp*	595
	Limonomyces sp	297	*Marasmius capillaris*	713	*Limonomyces sp*	321	*Limonomyces sp*	352	*Limonomyces sp*	278
	Trichoderma citrinoviride	240	*Trichoderma citrinoviride*	197	*Conocybe apala*	294	*Leptosphaerulina sp*	271	*Calvatia gigantea*	197
	Vascellum spNY_2013a	200	*Peziza varia*	173	*Trichoderma citrinoviride*	265	*Trichoderma citrinoviride*	237	*Trichoderma citrinoviride*	146
	Cristinia helvetica	151	*Limonomyces sp*	148	*Marasmius capillaris*	129	*Typhula maritima*	138	*Leptosphaerulina sp*	87

Table 4.5S cont.

Leptosphaerulina sp	117	*Lycoperdon pyriforme*	134	*Clitopilus sp*	91	*Clitopilus sp*	131	*Conocybe apala*	82
Drechslera spBAFC3419	89	*Leptosphaerulina sp*	53	*Leptosphaerulina sp*	82	*Peziza varia*	78	*Marasmius capillaris*	81
Peziza varia	89	*Pholiotina dentatomarginata*	50	*Cyathus striatus*	79	*Marasmius capillaris*	65	*Vascellum spNY_2013a*	71
Clitopilus sp	57	*Conocybe apala*	37	*Athelia bombacina*	74	*Agrocybe pediades*	60	*Clitopilus sp*	59

CHAPTER V

COUPLED CARBON AND NITROGEN INPUTS INCREASE MICROBIAL BIOMASS AND ACTIVITY IN PRAIRIE BIOENERGY SYSTEMS

A paper submitted to *Ecosystems*

Elizabeth M. Bach and Kirsten S. Hofmockel

Abstract

Soil microorganisms drive accrual and storage of soil carbon (C) and nitrogen (N) through decomposition of plant root and litter inputs. However, microbial activities vary greatly in time and space as well as with land management. The goal of this study was to address the seasonal role of microbial activity in soil C and N storage and cycling in harvested prairie and corn ecosystems. We measured extracellular enzyme activity, microbial biomass, extractable soil C and N, and total soil C and N at monthly intervals across two growing seasons in fertilized and unfertilized planted tallgrass prairie and compared them with a continuous *Zea mays* (corn) row-crop agroecosystem. Prairie systems supported greater microbial biomass and enzyme activity compared with corn systems; fertilized prairie systems had greater microbial activity than unfertilized prairies. The magnitude, and in some cases direction, of differences in response variables among the three managed systems changed seasonally. Overall, microbial biomass and enzyme activity were observed to be C-limited in corn systems and N-limited in unfertilized prairies. Furthermore, seasonal fluctuation in microbial activity underscores the importance of considering when soils are sampled to estimate and predict patterns in microbially-driven C and N cycling at the ecosystem level.

Keywords: tallgrass prairie, restoration, extracellular enzymes, microbial biomass, agroecosystem, N-acetyl-glucosaminidase, β-glucosidase

Introduction

Soils of central North America are among the most fertile in the world (Eswaran *et al.*, 2003), supporting plant production and wildlife in both tallgrass prairies and row-crop agroecosystems throughout the region. This soil fertility is derived in part through the activity of soil microorganisms recycling nutrients and energy. In the 10,000 years since the last glaciation, native grassland inputs and microbially-mediated cycling has built tremendous stores of carbon (C) in these soils. The use of these soils for arable crop production since the mid-1800s has stimulated microbial mineralization of native soil organic matter pools and substantially reduced soil C stocks (Matson et al., 1997; Stevenson and Cole, 1999). Additionally, inorganic nutrient run-off and soil erosion have dramatically impacted waterways within the region and beyond (Carpenter, 2005; Renwick *et al.*, 2008; Heathcote *et al.*, 2013). One approach to address these environmental concerns is to recouple soil microorganisms and plant inputs in managed ecosystems to build soil C and nitrogen (N) pools.

Soil microorganisms decompose plant tissues, releasing inorganic C and N. Increased belowground allocation of C by plants stimulates the abundance and activity of microorganisms, resulting in greater processing of C substrates and soil organic matter (Angers & Caron, 1998; DeGrood *et al.*, 2005). Previous studies suggest that microbial biomass can contribute 50% of the extractable soil organic C fractions and 80% of soil N

(Simpson *et al.*, 2007). In addition to biomass inputs, microorganisms facilitate decomposition of plant detritus and subsequent cycling of soil organic matter through production of extracellular enzymes. Microbes obtain nutrients and C substrates by excreting enzymes into their environment, and assimilating the products of the enzymatic reaction. The activities of multiple enzymes can be integrated to examine feedbacks between the environment and microbial metabolism driving C and N cycling in soils (Sinsabaugh *et al.*, 2009).

Generalizing about microbial activity at the ecosystem scale requires consideration of seasonal variation in temperature and precipitation (Wardle, 1998; Waldrop & Firestone, 2006; Wallenstein *et al.*, 2009; Yao *et al.*, 2011). Metabolic changes are driven in part by fluctuations in the abiotic soil environment, including freeze/thaw cycles, precipitation events, and drought. Biotic factors such as plant growth and root exudation also vary temporally, with high inputs in the warm growing season and essentially no inputs during winter in temperate regions. Together, seasonal changes in abiotic and biotic conditions drive temporal fluctuations in microbially-driven decomposition (Wardle, 1998; Williams & Rice, 2007; Bell *et al.*, 2010; Baldrian *et al.*, 2013; Koranda *et al.*, 2013). Seasonal variation in microbial activity can impact estimates of ecosystem C and nutrient pools and fluxes, which may include data from a variable number of sampling dates.

Estimates of C and N cycling and storage are crucial to evaluating agroecosystem alternatives that sustain and rebuild soil fertility in central North America. Ongoing research at the Comparison of Biofuel Systems (COBS) experiment in central Iowa is testing the hypothesis that reconstructed prairie ecosystems can be managed for

bioenergy stock production as a way to optimize ecological and economic benefits and costs in contrast with current row-crop systems. Overall, management of planted prairie for bioenergy production is showing promise for meeting ecological sustainability goals and potentially generating economic income for landowners (Jarchow & Liebman, 2012). Management of the planted prairie with moderate nitrogen (N) fertilization increases aboveground plant biomass (Jarchow & Liebman, 2013), but root biomass is greater in unfertilized prairie (Dietzel *unpublished data*). In comparison to corn agroecosystems, both prairie systems have minimal export of dissolved N and C in subsurface water flow, indicating the prairies with or without fertilizer retain water-soluble nutrients and C (Daigh, 2013). Prairie systems have greater total soil respiration (Daigh *et al.*, in press), but additional work indicates this may be driven by root and not microbial respiration (Nichols, 2014). However, the soil microbial mechanisms driving C and N accrual in these soils are not well understood.

Our goal was to assess the seasonal role of microbial activity in soil C and N storage and cycling in planted prairie ecosystems managed for sustainable bioenergy production and retention of soil C and nutrients. We measured extracellular enzyme activity, microbial biomass, extractable soil C and N, and total soil C and N at monthly intervals across two growing seasons in three bioenergy management systems at the COBS site: reconstructed diverse tallgrass prairie, fertilized reconstructed tallgrass prairie, and continuous *Zea mays* L. (corn) monoculture. We hypothesized that enzyme activity, biomass, and soil C and N pools would be lowest in corn row-crop agroecosystems and highest in unfertilized planted prairie, following root biomass inputs (Dietzel *unpublished data*). We further predicted that extracellular enzyme activity

would peak in mid-season, when temperatures and plant inputs were greatest in all ecosystems.

Materials & Methods

Study site

Soil was collected from the Iowa State University Comparison of Biofuel Systems (COBS) experimental site located on the South Reynoldson Farm in Boone County, IA (41°55'14.42"N, 93°44'58.96"W); see Jarchow and Liebman (2013) for a detailed site description. We sampled three experimental management systems: no-till continuous corn (*Zea mays*), planted tallgrass prairie, and fertilized planted tallgrass prairie. Both prairie systems were planted in 2008 with the same seeding mixture of 31 native species. Four replicate blocks contained four plots (27 m x 61 m) of each planting treatment in a randomized complete block design. Each plot was sampled monthly through the 2011 and 2012 growing season. Soils consisted of loams in the Nicollet (Fine-loamy, mixed, superactive, mesic Aquic Hapludoll) and Webster (Fine-loamy, mixed, superactive, mesic Typic Endoaquoll) series with less than 3% slope. Sand content ranged from 27% to 53% across the site, and clay content was 17% to 32%. In the five years before sampling, average growing season precipitation at the site was 91.8 cm and mean annual temperature was 9°C. All systems are rain-fed and receive no irrigation. Precipitation in our first sampling year, 2011, was wetter than average in spring with average temperature and precipitation late in the growing season. In 2012, precipitation was only 50% of the 60 year mean and temperatures were well above average, resulting in a record drought.

Soil sampling

Soils were sampled from the top 10 cm of soil using a slide-hammer soil coring device (5.5 cm diameter; Giddings Machine Company, Windsor, CO). Intact soil cores were placed in plastic bags, stored on ice, and transported to the laboratory. Three cores were collected from each plot at each sampling time. The mass of each individual core was used to calculate bulk density. Each core was gently broken up along natural points of weakness and passed through an 8-mm sieve, removing large roots and pebbles. Replicated cores were combined into one composite sample for each plot. A sub-sample of soil was removed immediately and dried at 105°C for 24 hours to determine field fresh gravimetric water content. The remaining soil was dried at 4°C in closed, sterilized plastic containers with desiccant until soil reached approximately 10% gravimetric water content (GWC, 2-4 days) as part of a larger experiment (Bach & Hofmockel, 2014; Bach and Hofmockel in review). Soil sub-samples were removed from this "cold dried" soil for subsequent analysis of microbial biomass C and N, extracellular enzyme activity, and total soil C and N. Microbial biomass was analyzed within 24 hours of sub-sampling. Sub-samples for extracellular enzyme activity were frozen immediately (-20° C). Sub-samples for total C and N were dried at 65°C for 4-5 days and ground before analysis.

Microbial biomass & activity

Microbial biomass C and N were determined using the chloroform fumigation-extraction method (Brookes *et al.*, 1985). Two 15g sub-samples of cold dried soil were utilized: one sample extracted with 0.5-M potassium sulfate (K_2SO_4) immediately and the second sample fumigated with chloroform ($CHCl_3$) for 24 hours and then extracted with

0.5-M K_2SO_4 after $CHCL_3$ was evacuated from the samples. Non-fumigated samples were also used to determine extractable C and N pools. Extracts were filtered through Whatman #42 filters, pre-leached with 0.5-M K_2SO_4 and frozen until analysis by wet-combustion on a TOC-L analyzer (Shimadzu Corporation, Columbia, MD, USA).

Extracellular soil enzyme assays were modified from Marx *et al.* (2001b) and DeForest (2009). Briefly, 1 g of frozen soil aggregates was suspended in 125 mL sodium acetate buffer with pH adjusted to median of soils (range 6.4-7.6). Slurries were pipetted into 96-well black micro-plates, and enzyme activities were determined by adding 4-Methylumbelliferyl (MUB)-linked substrates for N-acetyl-glucosaminidase (NAG) and β-glucosidase (BG), β-xylosidase (BX), and cellobiohydrolase (CB) for a final concentration of 40 mM. Assays were incubated in the dark for two hours, reactions stopped with 10μL 0.5 M NaOH, and solution optical density determined fluorometrically at 450 nm on a microplate reader (BioTek, Winooski, VT). In 2012, slight modifications were made to the enzyme assay protocol to reduce variability between analytical replicates. Enzyme concentrations were increased to 400 μM and soil-enzyme slurries were incubated in 5 mL tubes before being transferred to 96-well plates for fluorometric analysis. Re-analysis of a subset of 2011 samples with the modified protocol showed no changes in relationships detected in the original dataset. Original 2011 enzyme activity was greater than rerun samples, indicating enzyme activity had degraded in the additional 18 months of storage at -20° C, so we proceeded with the original 2011 and 2012 datasets despite changes made in the assay protocol, and no direct statistical comparisons were made between the 2011 and 2012 data. For all samples,

absolute potential enzyme activity (nmol h^{-1} g^{-1} dry aggregates) was calculated and reported as described by (German *et al.*, 2011).

Total soil C and N

Total soil C, and N, was determined for each sampling date. A sub-sample of soil was dried at 60°C for 48-60 hours, ground to a fine powder and dry combusted in a Thermo Flash 1112 CN analyzer (Thermo Corp, Lakewood, NJ). Total soil C and N was calculated as g C kg^{-1} dry soil and on an areal basis (g C m^{-2}) using soil bulk density.

Statistics

Microbial biomass, extracellular enzyme activities, and soil C and N pools from 2011 and 2012 were analyzed independently using a 2-way mixed model ANOVA with ecosystem and sampling month and their interaction as main factors (α=0.05). Block was included as a random factor and analyses were run using proc mixed in SAS v. 9.3. Data were natural log transformed to meet assumptions of normal distribution when necessary.

Results

Soil physical measures

Soil bulk density and moisture in the top 10 cm varied across the growing season in both 2011 and 2012. In 2011, soil bulk density varied independently between management systems and sampling dates. Soils in corn plots were more dense than prairies ($F_{2,33}$=10.44, P=0.0003) while across all soils June was the most dense and July the least dense ($F_{3,33}$=95.01, P<0.0001; Table 5.1). In 2012, bulk density was affected by

an interaction between sampling date and ecosystem ($F_{8,42}$=2.65, P=0.02). Corn plots had greatest soil bulk density in May and September, and both prairie systems were least dense in May and most dense in July.

The direction and magnitude of seasonal changes in soil moisture were ecosystem dependent in 2012 ($F_{8,42}$=6.69, P<0.0001; Table 5.1). In both years, corn plots had the highest soil moisture in May and lowest in August (2011) and September (2012) to 10-cm depth. Conversely, soil moisture within prairie systems was lowest in May and highest in June (2011) and July (2012). It should be noted that all soils also reached maximum gravimetric water content in October of 2012, which reflects increased precipitation in that month.

Soil C & N pools

Total soil C (TC) and total soil N (TN; g^{-1} soil) was affected by management system only and was greater in fertilized prairies than continuous corn in both sampling years ($P \leq 0.02$ for all; Table 5.2). Differences in TN and TC between management systems disappeared when adjusted for bulk density (Table 5.1), as lower bulk density in prairie systems reduces the areal soil mass and subsequently calculated C and N pools in the top 10 cm. Therefore, areal TC and TN pools fluctuated between sampling dates reflecting changes in soil bulk density (TC: $F_{3,30}$=3.95, P=0.02;TN: $F_{3,29}$=7.17, P=0.01). Expressing soil C and N pools on an areal basis is a common approach to compare between ecosystems, however, soil bulk density appears to be a dynamic soil property which is often overlooked, and can greatly influence ecosystem interpretations (Lee *et al.*, 2009).

Extractable C and N varied among management systems and across sampling months. Continuous corn had 25% more extractable N than fertilized prairie and 38% more than unfertilized prairie ($F_{2,35}$=20.19, P=0.0001; Table 5.2). Fertilized prairie had 33% more extractable C than unfertilized prairie, and corn plots were intermediate ($F_{2,31}$=3.30, P=0.05, Table 5.2). Extractable C was greatest in October and least in August for all systems ($F_{3,31}$=3.81, P=0.02). All relationships held true when expressed per gram dry soil and on an areal basis (m^{-2}), corrected for soil bulk density.

Microbial biomass & activity

Microbial biomass C (MBC) responded independently of both management system and sampling date (Fig. 5.1). Continuous corn had half the MBC of prairie ($F_{2,31}$=30.23, P<0.001, Fig. 5.1A), and in all management systems MBC was greatest in October ($F_{3,31}$=7.12, P<0.001, Fig. 5.1B). Microbial biomass N (MBN) exhibited an interaction between management system and sampling month, driven by a greater reduction in MBN in September in fertilized prairie than in corn and unfertilized prairie ($F_{6,35}$=2.49, P=0.04; Fig. 5.1C). When reported on an areal basis (multiplied by soil bulk density), the same relationships were present for both MBC and MBN. Increased soil moisture and post-harvest plant inputs likely explains maximum MBC and MBN in October. Differential response of MBC and MBN led to a significant interaction of management system and sampling date for MBC:MBN ratio ($F_{6,31}$=2.37, P=0.05; Fig. 5.1D). In September, MBC:MBN ratios were 100% greater in fertilized prairies, 30% in unfertilized prairies, and 55% greater in continuous corn than other sampling months.

Independent analysis of extracellular enzyme activity in the 2011 and 2012 growing seasons showed different trends in month to month enzyme activity trajectories (Fig. 5.2). In 2011, NAG and BG enzyme activity in both prairie treatments peaked in July and decreased in August, but increased throughout the growing season in continuous corn (NAG: $F_{6,29}$=3.04, P=0.02; BG: $F_{6,28}$=3.09, P=0.02). Potential BX activity was greater in fertilized prairie than corn, and overall activity was greatest in August and least in May (System: $F_{2,29}$=7.43, P=0.002, Date: $F_{3,29}$=8.39, P=0.0004). CB activity was affected by sampling month only; greatest activity occurred in August for all ecosystems ($F_{3,27}$=5.87, P=0.003). Separate analysis of 2012 soil showed no statistically significant seasonal trends in enzyme activity (Fig. 5.2). Large variance among biological replicates during the drought season of 2012 obscured intra-annual trends reflective of those observed in 2011. However, in both years there was a main effect of management system in which fertilized prairie had greater potential activity than continuous corn ($P \leq 0.04$ for all). For C-cycling enzymes BG, BX, and CB potential activity in fertilized prairies also exceeded potential activity in unfertilized prairies ($P \leq 0.03$ for all). Enzyme activity was also normalized for soil clay content because enzymes can be stabilized on clay particles, and all relationships remained similar to the potential activities reported above.

Microbial biomass specific enzyme activity in 2012 was greater in continuous corn than unfertilized prairie, but not fertilized prairie for BG ($F_{2,30}$=5.03, P=0.01). Other C-cycling enzymes, CB and BX, exhibited an interaction between management system and sampling date. This was driven by the high enzyme activities in August within fertilized prairie, which did not correspond with an increase in microbial biomass. Microbes may be responding to drought intensification by ramping up C-degrading

enzyme activity as labile root exudates diminished or packaging these enzymes in extracellular polysaccharide layers to protect against desiccation. Mass specific activity of NAG was constant across management systems and sampling dates. Together these data indicate microbes in soils under continuous corn are investing energy into enzyme production rather than growth. Greater root biomass and potentially associated root exudates are likely supporting greater microbial biomass and absolute enzyme activity in prairie systems. The variance among corn plots was greater than in prairies as well for all enzymes, which is consistent with the more heterogeneous distribution of plant inputs within and between corn rows.

Discussion

We found evidence of seasonal variation in extracellular enzyme activity and soil C and N pools, but seasonal dynamics varied among continuous corn, prairie, and fertilized prairie. The magnitude, and in some cases direction, of differences within the three management systems in the response variables changed with sampling dates. Overall, both prairies had greater microbial biomass and enzyme activity than continuous corn systems, consistent with previous findings (Baer *et al.*, 2010; Bach *et al.*, 2012). However, fertilized prairie had greater enzyme activity than unfertilized prairie, despite greater root biomass in unfertilized prairie (Dietzel *unpublished data*). Seasonal fluctuation in precipitation likely contributed to variation in microbial responses; however, the fact seasonal responses varied among management systems indicates that additional factors, which varied among the systems, also played a role.

Observed patterns in these data are consistent with a switch from microbial C-limitation in corn systems to N-limitation in unfertilized prairies. Corn cropping systems have very high inputs of inorganic N, but relatively low inputs of root biomass (Russell *et al.*, 2009). Furthermore, in our corn treatment, 50% of aboveground litter (stover) is harvested for bioenergy feedstock. Although aboveground biomass is also harvested in both prairie treatments, very low root biomass in corn likely limit C substrates available to soil microorganisms in corn (Hobbie & Hobbie, 2013; Kamble & Bååth, 2014), restricting microbial biomass in corn systems compared to perennial and native grassland ecosystems (Baer *et al.*, 2010; Jangid *et al.*, 2010). High microbial biomass-specific activity as observed in our study is further evidence that microbes in corn systems, especially those managed with stover removal, are likely C-limited, as microbes invest more energy per unit biomass into enzyme production than growth and reproduction (Schimel & Schaeffer, 2012; Hargreaves & Hofmockel, 2014). Data from N addition gradients in corn agroecosystems indicate greater N fertilization rates have minimal effect on soil organic C pools beyond increasing plant litter inputs, indicating microbes are limited by C inputs and not N (Brown *et al.*, 2014).

Unfertilized prairie exhibited release from C limitation with substantially greater microbial biomass and extracellular enzyme activity than continuous corn. However, greater enzyme activity and similar trend in microbial biomass in fertilized prairie indicates microbial growth and activity is further stimulated by N addition coupled with high root inputs, indicating C and N maybe co-limiting. The stoichiometry of nutrient availability in soil can greatly influence extracellular enzyme activity and decomposition rates of C substrates including cellulose and lignin (Sinsabaugh *et al.*, 2002; Sinsabaugh

& Follstad Shah, 2011). Addition of inorganic N has been found to generally suppress extracellular enzyme activity (Sinsabaugh *et al.*, 2002; Ramirez *et al.*, 2012), but these studies address direct effects of inorganic N without consideration of indirect effects, which have been shown to increase hydrolytic enzyme activity (Keeler *et al.*, 2009). In the prairies examined in this study, N addition increased presence of cool season (C_3) plants in fertilized prairies (Jarchow & Liebman, 2013), which has been shown to affect both the quality and timing of litter and root inputs (Craine *et al.*, 2002; Dornbush, 2007). Overall, coupled N availability and root inputs in fertilized prairies increased both microbial biomass and total enzyme activity, but resulted in lower microbial biomass-specific enzyme activity compared with continuous corn.

Seasonal fluctuations in microbial biomass and enzyme activities also reflect differential resource limitation among these management systems. Lower seasonal fluctuation in microbial biomass and enzyme activities in continuous corn compared with both prairie systems is consistent with plant-derived C-limitation. As plant inputs increased across the growing season, enzyme activity in continuous corn increased, but not microbial biomass, indicating microbes are investing metabolic resources into C-acquiring extracellular enzymes rather than growth (Hargreaves & Hofmockel, 2014). Relatively large swings in microbial biomass N, but not microbial biomass C in fertilized prairies is consistent with previous work showing microbes utilize added N without concomitant increases in C (Baer & Blair, 2008; Bell *et al.*, 2010). In the sort-term, microbes may allocate increased N to enzyme production rather than concomitant growth, and our observed seasonal fluctuations in enzyme activity throughout the growing season likely reflect indirect effects of N addition on plant inputs. Mid-summer

peaks in enzyme activity in fertilized prairie systems (July), may have been driven by senescence of C_3 plants, whereas peak enzyme activities late in the growing season, seen in both prairie systems, was likely driven by senescence of C_4 plants. Lack of differences in enzyme activity between the prairies in May indicated microbial activity responded more strongly to plant phenological changes than direct uptake of inorganic N, which was applied prior to the May sampling date. Inconsistent seasonal responses between the treatments further indicates plants play a role in regulating enzyme activity beyond environmental changes such as soil moisture, which varied consistently among the management systems.

Greater microbial biomass and activity in fertilized prairie appears to enhance accrual of soil C and N compared with unfertilized prairies, even after only three and four years since establishment (Table 5.2). Soil C accrual requires a balance of microbial growth and respiration which can be influenced by changes in the quantity and form of C and N inputs (Kuzyakov *et al.*, 2000). C additions has been shown to increase soil organic C stocks by increasing microbial biomass and activity in incubations (Bradford *et al.*, 2008) and field studies (Baer *et al.*, 2003; Bradford *et al.*, 2013). Previous work on prairie established on former agricultural fields provides evidence that microbes mineralize N more rapidly with N enrichment than with ambient or reduced N, reflecting a positive N cycling feedback (Baer & Blair, 2008). Our data support this view in that C- and N-cycling extracellular enzyme activity is enhanced by N addition, and that enhanced activity appears to lead to greater cycling of N through microbial and plant tissue (Jarchow & Liebman, 2013), recoupling N inputs with C. Although microbial respiration was not measured in this study, C and N inputs in the fertilized prairie appear to facilitate

microbial growth and organic matter stabilization rather than enhanced mineralization. This supports previous empirical and modeling work showing land transitioned from row-crop agricultural to grassland systems managed for bioenergy production can be a net C sink (Robertson *et al.*, 2011; Gelfand *et al.*, 2013).

In conclusion, we found that fertilizing reconstructed prairies generated greater microbial biomass and extracellular enzyme activity, effectively stabilizing and accruing soil organic matter. Seasonal fluctuations in enzyme activity were most dynamic in fertilized prairies and differences among the three bioenergy management systems varied widely between sampling dates. This finding underscores the importance of the timing and frequency with which soils are sampled to estimate and predict microbially-driven C and N cycling. Our observations from organic-rich mollisols in central Iowa support previous work indicating management of reconstructed prairies for bioenergy production shows promise for harnessing microbial communities to meet ecological goals of C storage and N retention in surface soils (Tilman *et al.*, 2006; Robertson *et al.*, 2011; Gelfand *et al.*, 2013).

Acknowledgements

Funding was provided by Agriculture and Food Research Initiative of the USDA National institute of Food and Agriculture (NIFA), grant number # 20116700330364 to KSH and an Iowa State University Plant Science Institute Fellowship to EMB. Giselle Narvaez, Becca Luzbetak, Kira Murray, Erin Frankson, Austin Putz, Eric Asbe, Megan Barthalomew, Dade McBrayer, and Mark Anthony provided assistance in the field and

laboratory. Sarah Hargreaves, Thomas Isenhart, Alison King, and Ryan Williams provided valuable feedback on drafts of the manuscript.

References

Angers DA, Caron J. 1998. Plant-induced changes in soil structure: Processes and feedbacks. Biogeochemistry 42: 55-72.

Bach EM, Baer SG, Six J. 2012. Plant and Soil Responses to High and Low Diversity Grassland Restoration Practices. Environmental Management 49: 412-424.

Bach EM, Hofmockel KS. 2014. Soil aggregate isolation method affects measures of intra-aggregate extracellular enzyme activity. Soil Biology & Biochemistry 69: 54-62.

Baer SG, Blair JM. 2008. Grassland establishment under varying resource availability: A test of positive and negative feedback. Ecology 89: 1859-1871.

Baer SG, Blair JM, Collins SL, Knapp AK. 2003. Soil resources regulate productivity and diversity in newly established tallgrass prairie. Ecology 84: 724-735.

Baer SG, Meyer CK, Bach EM, Klopf RP, Six J. 2010. Contrasting ecosystem recovery on two soil textures: implications for carbon mitigation and grassland conservation. Ecosphere 1: art5.

Baldrian P, Šnajdr J, Merhautová V, Dobiášová P, Cajthaml T, Valášková V. 2013. Responses of the extracellular enzyme activities in hardwood forest to soil temperature and seasonality and the potential effects of climate change. Soil Biology and Biochemistry 56: 60-68.

Bell TH, Klironomos JN, Henry HAL. 2010. Seasonal Responses of Extracellular Enzyme Activity and Microbial Biomass to Warming and Nitrogen Addition. Soil Sci. Soc. Am. J. 74: 820-828.

Bradford MA, Fierer N, Reynolds JF. 2008. Soil carbon stocks in experimental mesocosms are dependent on the rate of labile carbon, nitrogen and phosphorus inputs to soils. Functional Ecology 22: 964-974.

Bradford MA, Keiser AD, Davies CA, Mersmann CA, Strickland MS. 2013. Empirical evidence that soil carbon formation from plant inputs is positively related to microbial growth. Biogeochemistry 113: 271-281.

Brookes PC, Landman A, Pruden G, Jenkinson DS. 1985. Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology and Biochemistry 17: 837-842.

Brown KH, Bach EM, Drijber RA, Hofmockel KS, Jeske ES, Sawyer JE, Castellano MJ. 2014. A long-term nitrogen fertilizer gradient has little effect on soil organic matter in a high-intensity maize production system. Global Change Biology 20: 1339-1350.

Carpenter SR. 2005. Eutrophication of aquatic ecosystems: Bistability and soil phosphorus. Proceedings of the National Academy of Sciences of the United States of America 102: 10002-10005.

Craine JM, Tilman D, Wedin D, Reich P, Tjoelker M, Knops J. 2002. Functional traits, productivity and effects on nitrogen cycling of 33 grassland species. Functional Ecology 16: 563-574.

Daigh A. 2013. Soil physical properties, soil carbon dioxide fluxes, and soil drainage dynamics of select bioenergy cropping systems. Agronomy: Iowa State University.

Daigh AL, Sauer TJ, Xiao X, Horton R. in press. Spatial and temporal dynamics of soil-surface CO2 emissions in bioenergy cron rotations and reconstructed prairies. Soil Science Society of America Journal.

DeForest JL. 2009. The influence of time, storage temperature, and substrate age on potential soil enzyme activity in acidic forest soils using MUB-linked substrates and L-DOPA. Soil Biology & Biochemistry 41: 1180-1186.

DeGrood SH, Claassen VP, Scow KM. 2005. Microbial community composition on native and drastically disturbed serpentine soils. Soil Biology & Biochemistry 37: 1427-1435.

Dornbush ME. 2007. Grasses, litter, and their interaction affect microbial biomass and soil enzyme activity. Soil Biology & Biochemistry 39: 2241-2249.

Eswaran H, Beinroth FH, Reich PF. 2003. A global assessment of land quality. Cheltenham: Edward Elgar Publishing Ltd. 111-132p.

Gelfand I, Sahajpal R, Zhang X, Izaurralde RC, Gross KL, Robertson GP. 2013. Sustainable bioenergy production from marginal lands in the US Midwest. Nature 493: 514-517.

German DP, Weintraub MN, Grandy AS, Lauber CL, Rinkes ZL, Allison SD. 2011. Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Biology & Biochemistry 43: 1387-1397.

Hargreaves SK, Hofmockel KS. 2014. Physiological shifts in the microbial community drive changes in enzyme activity in a perennial agroecosystem. Biogeochemistry 117: 67-79.

Heathcote AJ, Filstrup CT, Downing JA. 2013. Watershed Sediment Losses to Lakes Accelerating Despite Agricultural Soil Conservation Efforts. PLoS ONE 8: e53554.

Hobbie JE, Hobbie EA. 2013. Microbes in nature are limited by carbon and energy: the starving-survival lifestyle in soil and consequences for estimating microbial rates. Frontiers in Microbiology 4.

Jangid K, Williams MA, Franzluebbers AJ, Blair JM, Coleman DC, Whitman WB. 2010. Development of soil microbial communities during tallgrass prairie restoration. Soil Biology and Biochemistry 42: 302-312.

Jarchow ME, Liebman M. 2012. Tradeoffs in biomass and nutrient allocation in prairies and corn managed for bioenergy production. Crop Science 52: 1330-1342.

Jarchow ME, Liebman M. 2013. Nitrogen fertilization increases diversity and productivity of prairie communities used for bioenergy. GCB Bioenergy 5: 281-289.

Kamble PN, Bååth E. 2014. Induced N-limitation of bacterial growth in soil: Effect of carbon loading and N status in soil. Soil Biology and Biochemistry 74: 11-20.

Keeler BL, Hobbie SE, Kellogg LE. 2009. Effects of Long-Term Nitrogen Addition on Microbial Enzyme Activity in Eight Forested and Grassland Sites: Implications for Litter and Soil Organic Matter Decomposition. Ecosystems 12: 1-15.

Koranda M, Kaiser C, Fuchslueger L, Kitzler B, Sessitsch A, Zechmeister-Boltenstern S, Richter A. 2013. Seasonal variation in functional properties of microbial communities in beech forest soil. Soil Biology and Biochemistry 60: 95-104.

Kuzyakov Y, Friedel JK, Stahr K. 2000. Review of mechanisms and quantification of priming effects. Soil Biology and Biochemistry 32: 1485-1498.

Lee J, Hopmans JW, Rolston DE, Baer SG, Six J. 2009. Determining soil carbon stock changes: Simple bulk density corrections fail. Agriculture Ecosystems & Environment 134: 251-256.

Marx MC, Wood M, Jarvis SC. 2001. A microplate fluorimetric assay for the study of enzyme diversity in soils. Soil Biology and Biochemistry 33: 1633-1640.

Nichols V. 2014. Shedding light on CO2 emissions and fuel yields in maize and prairie biofuel systems. Agronomy: Iowa State University.

Ramirez KS, Craine JM, Fierer N. 2012. Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. Global Change Biology 18: 1918-1927.

Renwick WH, Vanni MJ, Zhang Q, Patton J. 2008. Water Quality Trends and Changing Agricultural Practices in a Midwest U.S. Watershed, 1994–2006. Journal of Environmental Quality 37: 1862-1874.

Robertson GP, Hamilton SK, Del Grosso SJ, Parton WJ. 2011. The biogeochemistry of bioenergy landscapes: carbon, nitrogen, and water considerations. Ecological Applications 21: 1055-1067.

Russell AE, Cambardella CA, Laird DA, Jaynes DB, Meek DW. 2009. Nitrogen fertilizer effects on soil carbon balances in Midwestern US agricultural systems. Ecological Applications 19: 1102-1113.

Schimel J, Schaeffer SM. 2012. Microbial control over carbon cycling in soil. Frontiers in Microbiology 3.

Simpson AJ, Simpson MJ, Smith E, Kelleher BP. 2007. Microbially Derived Inputs to Soil Organic Matter: Are Current Estimates Too Low? Environmental Science & Technology 41: 8070-8076.

Sinsabaugh R, Follstad Shah J. 2011. Ecoenzymatic stoichiometry of recalcitrant organic matter decomposition: the growth rate hypothesis in reverse. Biogeochemistry 102: 31-43.

Sinsabaugh RL, Carreiro MM, Repert DA. 2002. Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. Biogeochemistry 60: 1-24.

Sinsabaugh RL, Hill BH, Shah JJF. 2009. Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. Nature 462: 795-U117.

Tilman D, Hill J, Lehman C. 2006. Carbon-negative biofuels from low-input high-diversity grassland biomass. Science 314: 1598-1600.

Waldrop MP, Firestone MK. 2006. Seasonal dynamics of microbial community composition and function in oak canopy and open grassland soils. Microbial Ecology 52: 470-479.

Wallenstein MD, McMahon SK, Schimel JP. 2009. Seasonal variation in enzyme activities and temperature sensitivities in Arctic tundra soils. Global Change Biology 15: 1631-1639.

Wardle DA. 1998. Controls of temporal variability of the soil microbial biomass: A global-scale synthesis. Soil Biology and Biochemistry 30: 1627-1637.

Williams MA, Rice CW. 2007. Seven years of enhanced water availability influences the physiological, structural, and functional attributes of a soil microbial community. Applied Soil Ecology 35: 535-545.

Yao H, Bowman D, Shi W. 2011. Seasonal variations of soil microbial biomass and activity in warm- and cool-season turfgrass systems. Soil Biology and Biochemistry 43: 1536-1543.

Figures

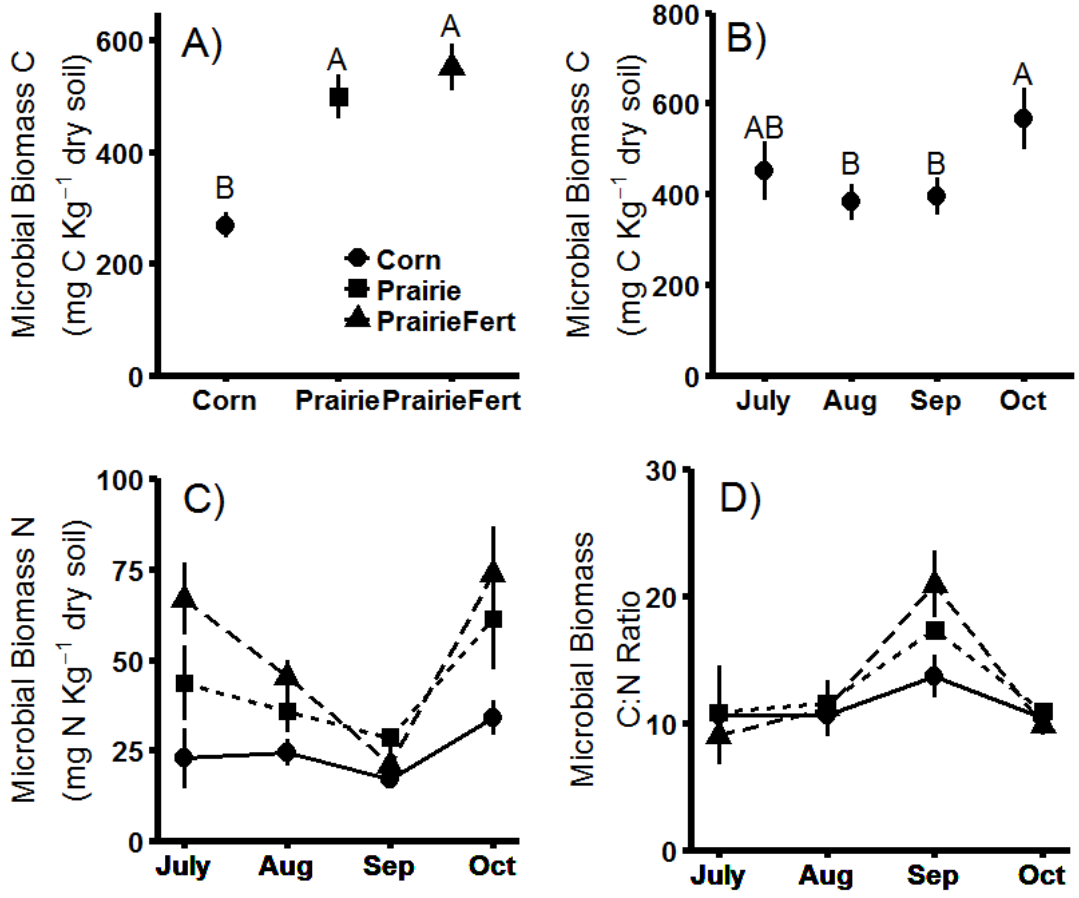

Figure 5.1: Microbial biomass carbon (C) among A) management system and B) sampling date. Management system and sampling date interaction in C) microbial biomass nitrogen (N) and D) the C:N ratio of microbial biomass. Circles represent continuous corn, squares represent unfertilized prairie, and triangles represent fertilized prairie. Letters indicate statistical main effect ($\alpha<0.05$). Symbols represent mean values ($n_{ecosystem}=20$, $n_{date}=12$, $n_{ecosystem*date}=4$).

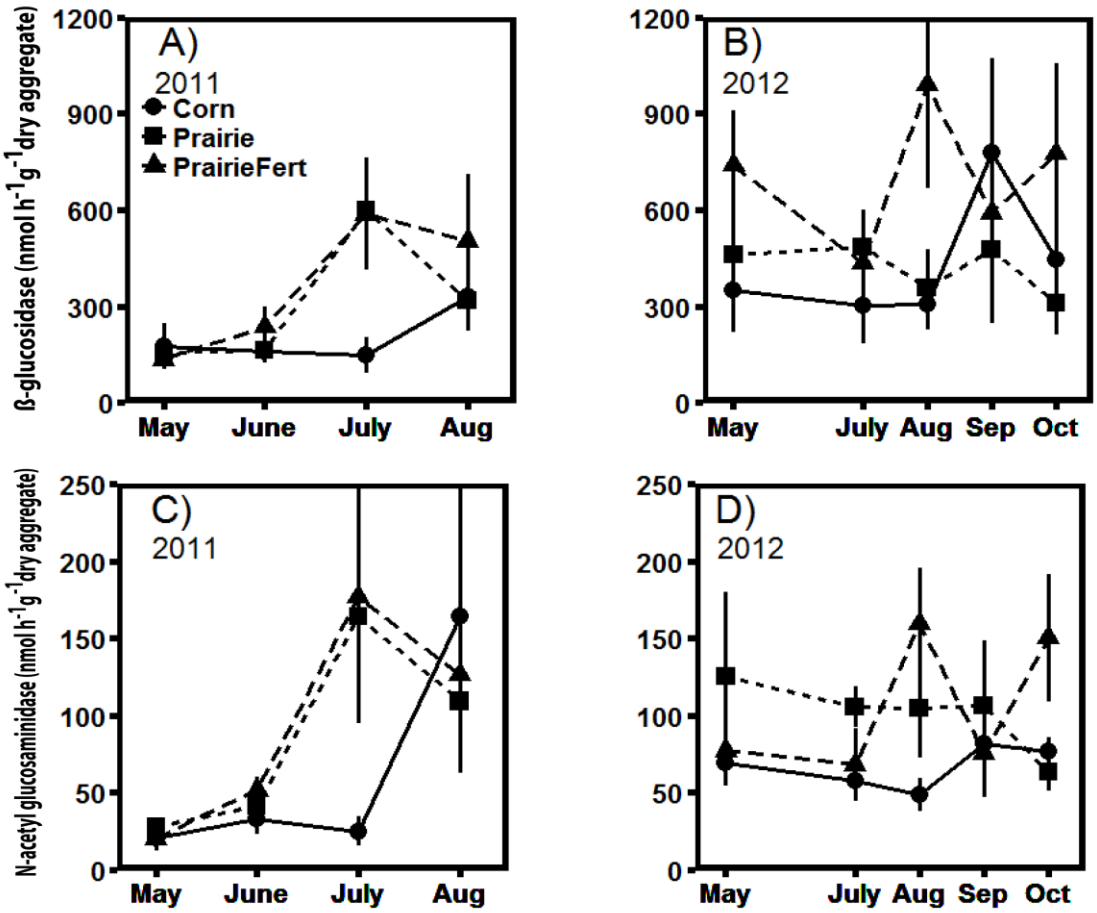

Figure 5.2: Potential extracellular enzyme activity for β-glucosidase (A, B) and N-acetyl glucosaminidase (C, D) across the 2011(A, C) and 2012 (B, D) growing seasons. Circles represent continuous corn, squares represent unfertilized prairie, and triangles represent fertilized prairie. Symbols represent mean values (*n*=4).

Tables

Table 5.1: Soil physical conditions (mean ±1 SE) from corn, fertilized prairie, and unfertilized prairie at each sampling date across two growing seasons (*n*=4). Soil temperature is average of temperature at 5 cm and 10 cm below soil surface. Different letters denote statistical difference between means of sampling date within an ecosystem in each year (α=0.05). Corn systems denoted in plain text, **fertilized prairie denoted in bold**, and *prairie denoted in italics*.

Management *Sampling date*	**Gravimetric Water Content**	**pH**	**Soil Temperature (°C)**	**Bulk Density (g soil cm^{-3})**
Continuous Corn				
2011-May	0.21±0.01	$6.5±0.1^{B}$	16.8±0.3	1.57±0.03
2011-June	0.12±0.01	$6.4±0.1^{B}$	18.6±0.3	1.66±0.03
2011-July	0.13±0.01	$6.6±0.1^{B}$	22.5±0.3	1.39±0.03
2011-August	0.18±0.01	$7.1±0.1^{A}$	21.5±0.3	1.43±0.03
2012-May	$0.167±0.009^{A}$	5.9^{B}	15.9±0.9	1.21±0.04
2012-July	$0.122±0.009^{BC}$	6.5^{AB}	21.8±0.2	1.16±0.04
2012-August	$0.113±0.009^{C}$	6.6^{A}	19.3±0.1	1.12±0.04
2012-September	$0.108±0.009^{C}$	6.7^{A}	13.9±0.6	1.24±0.04
2012-October	$0.158±0.009^{AB}$	6.6^{A}	.	1.22±0.04
Fertilized Prairie				
2011-May	0.19±0.01	7.2±0.1	14.1±0.1	1.51±0.03
2011-June	0.12±0.01	7.2±0.1	17.6±0.2	1.64±0.03

Table 5.1 continued

2011-July	0.16±0.01	7.1±0.1	20.8±0.1	1.26±0.03
2011-August	0.19±0.01	7.3±0.1	20.6±0.2	1.30±0.03
2012-May	0.112±0.009^{B}	7.1	16.0±0.3	0.89±0.04^{B}
2012-July	0.151±0.009 ^{AB}	7.4	21.3±0.3	1.10±0.04^{A}
2012-August	0.125±0.009^{B}	7.3	18.9±0.2	1.06±0.04^{AB}
2012-September	0.111±0.009 ^{B}	7.2	14.2±0.4	1.05±0.04^{AB}
2012-October	0.190±0.009^{A}	7.5	.	1.15±0.04^{A}
Unfertilized Prairie				
2011-May	0.19±0.01	7.4±0.1	15.1±0.2	1.54±0.03
2011-June	0.12±0.01	7.2±0.1	18.6±0.2	1.64±0.03
2011-July	0.15±0.01	7.0±0.1	23.2±0.3	1.27±0.03
2011-August	0.17±0.01	7.3±0.1	21.4±0.3	1.39±0.03
2012-May	0.100±0.009^{C}	7.6	17.3±0.4	0.99±0.04^{B}
2012-July	0.146±0.009^{AB}	7.4	22.9±0.4	1.20±0.04^{A}
2012-August	0.126±0.009^{BC}	7.3	20.1±0.4	1.13±0.04^{AB}
2012-September	0.110±0.009^{BC}	7.2	14.6±0.5	1.16±0.04^{AB}
2012-October	0.175±0.009^{A}	7.3	.	1.19±0.04^{A}

Table 5.2: Carbon (C) and nitrogen (N) pools in soils under continuous corn, fertilized prairie (PrairieFert), and unfertilized prairie. Values are mean of four sampling dates in 2011 and five sampling dates in 2012. Management system was a main effect and did not interact with sampling date for these variables. Different letters denote statistical difference between ecosystems (α=0.05).

	2011			2012		
	Corn	**Prairie**	**Prairie Fert**	**Corn**	**Prairie**	**Prairie Fert**
TC (g C Kg^{-1} soil)	21.92^{B*}	24.66^{AB*}	25.80^{A*}	21.95^{B*}	23.78^{AB*}	26.72^{A*}
SE	±1.69	±1.67	±1.71	±1.72	±1.72	±1.72
TN (g N Kg^{-1} soil)	1.74^{B*}	1.92^{AB*}	2.01^{A*}	1.76^{B*}	1.80^{AB*}	2.01^{A*}
SE	±0.12	±0.12	±0.13	±0.12	±0.12	±0.12
TC (Kg C m^{-2})	3.32	3.57	3.66	2.60	2.67	2.80
SE	±0.21	±0.21	±0.21	±0.17	±0.17	±0.17
TN (Kg N m^{-2})	0.26	0.28	0.29	0.21	0.20	0.21
SE	±0.02	±0.02	±0.02	±0.01	±0.01	±0.01
CN	12.58	12.91	12.84	12.48^{B**}	13.19^{A**}	13.23^{A**}
SE	±0.15	±0.15	±0.15	±0.16	±0.16	±0.16
Extractable C (μg C g^{-1} dry soil)				26.20^{AB*}	20.68^{B*}	28.65^{A*}
SE				±2.90	±2.90	±2.77
Extractable N (μg N g^{-1} dry soil)				8.37^{A***}	4.04^{B***}	4.77^{B***}

Table 5.2 continued

SE	±0.57	±0.55	±0.55
Extractable C (g C m^{-2})	3.03^{AB*}	2.43^{B*}	3.14^{A*}
SE	±0.31	±0.31	±0.30
Extractable N (g N m^{-2})	0.98^{A***}	0.48^{B***}	0.52^{B***}
SE	±0.07	±0.07	±0.07

CHAPTER VI

GENERAL DISCUSSION & CONCLUSIONS

Consideration of microbial ecology at the micro-scale contributes new insights into the role of soil physical structure on soil microorganisms driving ecosystem-level processes (Ritz, 2011). Utilization of the optimal sieving approach improved analysis biological communities and activities at a scale more relevant to microorganisms. Increased carbon (C) and nitrogen (N) content in large macroaggregates from plant-derived inputs increased N-cycling enzyme activity (N-acetyl glucosaminidase, chitinase) and abundance of the Basidiomycota family Strophariaceae. Microaggregates exhibited greater C-cycling enzyme activity (cellobiohydrolase) and fungal taxa richness. These observe micro-scale differences influenced overall ecosystem C and N cycling and storage

Differences in enzyme activity and fungal community structure across soil aggregate fractions provide a biological signal of habitat differences. Increased chitinase activity in large macroaggregates could be indicative of greater chitin and amino-sugar substrates, potentially generated by increased fungal hyphae stabilizing large macroaggregates. There was greater abundance of fungal sequences in large macroaggregates, but sequence abundance cannot be interpreted as directly indicative of biomass differences. Alternatively, increased plant-derived C substrates in large macroaggregates could increase microbial demand for organic N (Geisseler & Horwath, 2009), inducing an increase in chitinase production. Increased cellobiohydrolase activity in microaggregate fractions does contradict the prevailing paradigm that microaggregates

are enriched in chemically complex C molecules, not plant-derived cellulose (Jastrow, 1996; Six *et al.*, 2001). However, the optimal moisture approach isolates free micoraggregates, which are not occluded within macroaggregates. Microbes and enzymes are likely largely stabilized on the surface of microaggregates and interacting with organic matter in pore space surrounding the microaggregates . Thus, microaggregates may still occluded chemically recalcitrant C internally, as indicated by lower C:N ratio observed in microaggregates, and support processing of labile inputs on the surface. Stabilization of enzymes and microbes in microaggregate surfaces would also contribute to binding microaggregates together to form macroaggregates. The optimal moisture approach enables a more sensitive perspective on biological responses to soil aggregation, and it is not unexpected these signals differ somewhat from classic slaking approaches focused on detecting long-term patterns in organic matter chemistry. Data from both approaches can be used to further inform ecosystem C and N cycling on short- and long-term time scales.

Analysis of soil aggregates at multiple time points within growing seasons showed aggregate turn-over and reformation within weeks and increases in extracellular enzyme activity with large macroaggregate disruption. This empirical evidence supports the paradigm that large macroaggregates release organic matter when they disintegrate, increasing microbial access to substrate (Six *et al.*, 2000a). Reformation of large macroaggregates following peaks in enzyme activity also supports evidence that microbial activity plays a central role in macroaggregate formation and stabilization (Jastrow *et al.*, 1998; Denef *et al.*, 2002). Increased abundance of Strophariaceae in large macroaggregates, regardless of land use may indicate these fungi play a key role in large

macroaggregate formation and stabilization, and future research should target members of this family. This dissertation presents the first data from sequential field-collected aggregates supporting these ideas largely formed around incubation experiments.

In addition to soil aggregate turnover, phenology of belowground plant inputs also influenced extracellular enzyme activity and fungal communities. Each of the ecosystems exhibited unique seasonal patterns of extracellular enzyme activity, microbial biomass N, and fungal community structure. Under continuous corn, microbial biomass N and extracellular enzyme activity increased throughout the growing season as roots grew and senesced. In perennial prairie systems, extracellular enzyme activity and microbial biomass was greater overall as microbes interacted with root tissue for the entire year, even outside of the growing season. Fertilized prairie systems exhibited peak enzyme activity earlier in the summer than unfertilized prairie, likely driven by senescing C_3 plants, which were found in greater abundance in the fertilized prairie system (Jarchow & Liebman, 2013). Fungal community structure also shifted between July and October sampling dates in corn and fertilized prairie, but not in unfertilized prairie. This may be a recovery response from inorganic N addition earlier in the growing season. Nitrogen fertilization has been shown to affect fungal community structure in other systems (Nemergut *et al.*, 2008; Allison *et al.*, 2010; Weber *et al.*, 2013). Alternatively, this may also be a plant-driven seasonal response, as it is known grassland plant community structure, especially relative abundance of perennial, annual, C_3 and C_4 grasses can influence soil fungal communities, especially arbuscular mycorrhizal fungi (Hetrick *et al.*, 1988; Hiiesalu *et al.*, 2014).

Differences in aggregate distribution and plant inputs among the three bioenergy systems contributed to differences in ecosystem-level soil C and N cycling and storage. Increased enzyme activity in fertilized prairie systems increased microbial processing of plant-derived organic matter, but also facilitated aggregate formation which protected that organic matter from complete mineralization. This is despite unfertilized prairie producing more root biomass (Dietzel, 2014) and lower quality root tissue (Jarchow & Liebman, 2012). Thus, the role of soil microbial biomass and activity was greater than root biomass in driving soil aggregation in these systems. Low levels of microbial biomass and enzyme activity under corn may be a main contributor to lower aggregation even under no-till management. Microbial biomass and enzymatic production in corn was likely C-limited, due to limited root biomass and high available N (Hobbie & Hobbie, 2013; Hargreaves & Hofmockel, 2014).

The data presented in this dissertation have contributed to greater mechanistic understanding of the factors driving microbial community structure and activity from the micro-scale to the ecosystem-scale. In addition to these contributions to the field of soil ecology, these results have potential to shape research and applications leveraging microbes and microbial functioning in grasslands and cultivated lands to address local and regional environmental challenges. Agricultural regions, including Iowa and the central United States, are confronting numerous environmental challenges such as nutrient leakage from soils to waterways (Renwick *et al.*, 2008), soil erosion (Heathcote *et al.*, 2013), and release of greenhouse gasses (Johnson *et al.*, 2007; Foley *et al.*, 2011). Research indicates land management practices, including the bioenergy production

systems presented here, could be targeted to facilitate microbial responses to improve C and nutrient storage in soils.

Literature Cited

Allison, S. D., T. B. Gartner, M. C. Mack, K. McGuire, and K. Treseder. 2010. Nitrogen alters carbon dynamics during early succession in boreal forest. Soil Biology and Biochemistry **42**:1157-1164.

Denef, K., J. Six, R. Merckx, and K. Paustian. 2002. Short-term effects of biological and physical forces on aggregate formation in soils with different clay mineralogy. Plant and Soil **246**:185-200.

Dietzel, R. 2014. Comparing soil carbon storage potential in corn- and prairie-based cropping systems. Iowa State Univeristy.

Foley, J. A., N. Ramankutty, K. A. Brauman, E. S. Cassidy, J. S. Gerber, M. Johnston, N. D. Mueller, C. O/'Connell, D. K. Ray, P. C. West, C. Balzer, E. M. Bennett, S. R. Carpenter, J. Hill, C. Monfreda, S. Polasky, J. Rockstrom, J. Sheehan, S. Siebert, D. Tilman, and D. P. M. Zaks. 2011. Solutions for a cultivated planet. Nature **478**:337-342.

Geisseler, D. and W. R. Horwath. 2009. Relationship between carbon and nitrogen availability and extracellular enzyme activities in soil. Pedobiologia **53**:87-98.

Hargreaves, S. K. and K. S. Hofmockel. 2014. Physiological shifts in the microbial community drive changes in enzyme activity in a perennial agroecosystem. Biogeochemistry **117**:67-79.

Heathcote, A. J., C. T. Filstrup, and J. A. Downing. 2013. Watershed Sediment Losses to Lakes Accelerating Despite Agricultural Soil Conservation Efforts. PLoS ONE **8**:e53554.

Hetrick, B. A. D., D. G. Kitt, and G. T. Wilson. 1988. Mycorrhizal dependenc and growth habit of warm-season and cool-season tallgrass prairie plants. Canadian Journal of Botany-Revue Canadienne De Botanique **66**:1376-1380.

Hiiesalu, I., M. Pärtel, J. Davison, P. Gerhold, M. Metsis, M. Moora, M. Öpik, M. Vasar, M. Zobel, and S. D. Wilson. 2014. Species richness of arbuscular mycorrhizal fungi: associations with grassland plant richness and biomass. New Phytologist:n/a-n/a.

Hobbie, J. E. and E. A. Hobbie. 2013. Microbes in nature are limited by carbon and energy: the starving-survival lifestyle in soil and consequences for estimating microbial rates. Frontiers in Microbiology **4**.

Jarchow, M. E. and M. Liebman. 2012. Tradeoffs in biomass and nutrient allocation in prairies and corn managed for bioenergy production. Crop Science **52**:1330-1342.

Jarchow, M. E. and M. Liebman. 2013. Nitrogen fertilization increases diversity and productivity of prairie communities used for bioenergy. GCB Bioenergy **5**:281-289.

Jastrow, J. D. 1996. Soil aggregate formation and the accrual of particulate and mineral-associated organic matter. Soil Biology & Biochemistry **28**:665-676.

Jastrow, J. D., R. M. Miller, and J. Lussenhop. 1998. Contributions of interacting biological mechanisms to soil aggregate stabilization in restored prairie. Soil Biology & Biochemistry **30**:905-916.

Johnson, J. M. F., A. J. Franzluebbers, S. L. Weyers, and D. C. Reicosky. 2007. Agricultural opportunities to mitigate greenhouse gas emissions. Environmental Pollution **150**:107-124.

Nemergut, D. R., A. R. Townsend, S. R. Sattin, K. R. Freeman, N. Fierer, J. C. Neff, W. D. Bowman, C. W. Schadt, M. N. Weintraub, and S. K. Schmidt. 2008. The effects of chronic nitrogen fertilization on alpine tundra soil microbial communities: implications for carbon and nitrogen cycling. Environmental Microbiology **10**:3093-3105.

Renwick, W. H., M. J. Vanni, Q. Zhang, and J. Patton. 2008. Water Quality Trends and Changing Agricultural Practices in a Midwest U.S. Watershed, 1994–2006. Journal of Environmental Quality **37**:1862-1874.

Ritz, K. 2011. Architecture and Biology and Soils : Life in Inner Space. CABI, Wallingford, Oxfordshire, UK.

Six, J., E. T. Elliott, and K. Paustian. 2000. Soil macroaggregate turnover and microaggregate formation: A mechanism for C sequestration under no-tillage agriculture. Soil Biology & Biochemistry **32**:2099-2103.

Six, J., G. Guggenberger, K. Paustian, L. Haumaier, E. T. Elliott, and W. Zech. 2001. Sources and composition of soil organic matter fractions between and within soil aggregates. European Journal of Soil Science **52**:607-618.

Weber, C. F., R. Vilgalys, and C. R. Kuske. 2013. Changes in fungal community composition in response to elevated atmospheric CO2 and nitrogen fertilization varies with soil horizon. Frontiers in Microbiology **4**.